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COMMENTARY

WHAT IS THE NATURE OF MECAMYLAMINE'S ANTAGONISM OF THE CENTRAL EFFECTS OF NICOTINE?

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The presence of nicotine receptors in the CNS has been recognized for a long time. The evidence for central nicotine receptors was initially based upon the fact that acetylcholine served as the major neurotransmitter associated with the numerous central effects exerted by nicotine and that these effects were blocked by ganglionic antagonists. Interest has increased in the past decade as the result of *in vitro* binding studies which led to a more definitive characterization of nicotine receptors in the central nervous system. One of the most difficult aspects has been the determination of the functional significance of these binding sites. This latter point is no simple matter since nicotine exerts numerous effects on the central nervous system. However, it would seem likely that at least some of these binding sites play a key role in the expression of centrally mediated behavioral effects of nicotine. Indeed, it is thought that the interaction of nicotine with its binding sites in the CNS may be the major factor underlying the continued smoking habit [1, 2].

Antagonists have always played a major role in establishing the existence of a particular receptor. Actually, most central neurotransmitter receptors have been characterized by *in vitro* binding experiments performed with a radiolabeled antagonist rather than an agonist. Despite the fact that the pharmacological manipulations by antagonists were crucial in establishing nicotinic receptors, it remains unclear how these antagonists are interfering with the actions of nicotine. Considerable evidence is emerging that these antagonists are not acting in a simple competitive or noncompetitive fashion at the nicotine receptor. The objective of this commentary is to explore the nature of this antagonism by mecamylamine and related antagonists.

Antagonism of the central effects of nicotine

There is ample evidence that the central nicotinic cholinergic receptor is of the type that is blocked by non-depolarizing ganglionic blocking agents which include mecamylamine, pempidine, hexamethonium, pentolinium and trimethaphan. The effects of nicotine that are blocked by mecamylamine include: cardiovascular effects [3], hypothermia [4], ear twitching [5], tremors and antidiuresis [6], behavioral effects [7–10] and antinociception [11]. There are also numerous studies which demonstrate that pretreatment with mecamylamine influences cigarette smoking [12] as well as the physiological and subjective effects of nicotine [13]. Evidence that mecamylamine is acting centrally to block these effects of nicotine has come from several studies. Wu and Martin [3] found that mecamylamine, but not naloxone, would antagonize the cardiovascular effects produced by microinjections of nicotine into the nucleus ambiguus. Hall [4] has also shown that the hypothermic response to nicotine is abolished by the intraventricular injection of mecamylamine. Ear twitching in the cat induced by intraventricular injection of nicotine is blocked by mecamylamine [5]. Although it is thought that hexamethonium acts peripherally due to its relative inability to penetrate the CNS following systemic administration [14], the introduction of hexamethonium directly into the central nervous system (intraventricular administration, perfusion through the subarachnoid space, or direct application to the ventral surface of the medulla) results in blockade of many of the central effects of nicotine which include hypotension [15, 16], hypothermia, salivation and motor reflexes [4].

The mechanism by which mecamylamine exerts its peripheral anticholinergic effects has generally been attributed to competitive antagonism at the ganglia. However, the antagonistic properties of mecamylamine are undoubtedly more complex than simple competition at the ganglia. Indeed, van Rossum *et al.* [17] reported that mecamylamine and pempidine exhibit both competitive and noncompetitive antagonistic properties in blocking the stimulation by nicotine of frog rectus and guinea pig intestine. Lees and Nishi [18] concluded that mecamylamine's antagonism of nicotine in the rabbit superior cervical ganglion was due to both presynaptic and postsynaptic inhibitory actions. In addition, Blackman and Ray [19] demonstrated that high concentrations of mecamylamine and pempidine block the effect of nicotine at the neuromuscular junction of the diaphragm of the rat. Electrophysiological studies have also been used to gain insight into the mechanism of action of nicotine antagonists. Ascher *et al.* [20] showed that trimethapan and mecamylamine act in a competitive fashion on the submandibular ganglion of the rat, whereas hexamethonium acts mainly by blocking the channel when it is opened by a cholinergic agonist. Likewise, David and Sattelle [21] reported that

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mecamylamine is a competitive antagonist at the receptor/ion channel on motor neurons of the cockroach.

Although the general assumption has been that central nicotinic receptors are of the ganglionic type, there is some evidence for the existence of central receptors that resemble the peripheral neuromuscular cholinergic receptor. Nicotine excitation of neurons in the brain stem of cats and rats has been shown to be blocked by dihydro-β-erythroidine [22, 23], a non-charged competitive neuromuscular blocker. Also, Bradley and Lucy [24] demonstrated that iontophoretic application of nicotine to rat medulla results in excitation of respiratory neurons that is blocked by dihydro-β-erythroidine. Varanda *et al.* [25] recently reported that mecamylamine acts as a noncompetitive antagonist at the neuromuscular junction. These antagonism studies taken together strongly suggest that nicotine may interact with more than one receptor subtype in the central nervous system. Indeed, there is evidence that central nicotinic cholinergic receptors in chick brain are structurally distinct from peripheral nicotinic receptors [26].

It is not surprising that characterization of nicotine binding sites in brain tissue using radiolabeled neurotoxins which are vastly different in structure, acetylcholine and nicotine, would result in a lack of consensus as to the nature of the central nicotine receptor. There are several lines of evidence to suggest that there are multiple nicotine receptors in brain. Sershen *et al.* [27] have provided evidence that there is a noncholinergic nicotine receptor, whereas Eldefrawi *et al.* [28] showed that acetylcholine would displace approximately half of [^{3}H]nicotine binding and that curare would displace [^{3}H]acetylcholine binding to brain tissue. Other investigators [29–31] have shown that either acetylcholine or nicotinic agonists would compete effectively for [^{3}H]nicotine binding. Even more direct evidence has been provided by Schwartz *et al.* [32] who demonstrated that nicotine would displace [^{3}H]acetylcholine binding not displaced by diisopropylfluorophosphate and atropine. In addition to suggestions that there are cholinergic and non-cholinergic nicotine receptors, several investigators have provided direct evidence for more than one nicotine binding site in brain [31, 33, 34].

It is important to determine whether mecamylamine and the classical C-6 antagonists compete with the binding of nicotinic agonists, due to the fact that they effectively antagonize the central effects of nicotine. However, it has been amply demonstrated that antagonists do not compete for binding of any of the nicotine agonists (see Martin [35] for a review). Several explanations for this discrepancy have been presented, but none have been supported by scientific data. Romano *et al.* [29] suggested that the long incubations that were used in their studies resulted in an agonist-induced shift in the binding site to a high-affinity site that was agonist selective. In this way nicotine would prevent mecamylamine from binding to the receptor. Marks and Collins [30] proposed that nicotinic agonists and antagonists do not bind at the same sites. This latter suggestion seems to be more plausible. Also, Ascher *et al.* [20] have

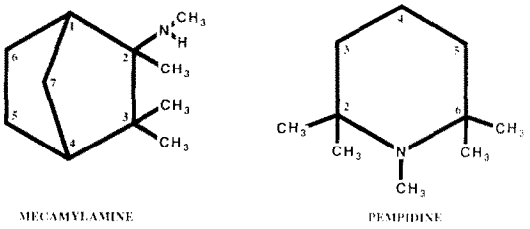

Fig. 1. Structures of mecamylamine and pempidine.

suggested that mecamylamine may be acting by blocking the ion channel associated with nicotinic receptors and that mecamylamine may be a noncompetitive antagonist. David and Sattelle [21] showed that mecamylamine acts in a voltage-independent manner in the motor neuron of the cockroach, suggestive of competitive antagonism at a closed channel, but this site differs from the one to which α-bungarotoxin binds. They concluded that binding studies may be a poor indicator of its actions on the receptor/ion channel complex.

While it has been assumed that the antagonists are interacting directly with the nicotinic receptor, there are several other possibilities. The antagonists could block the effects of nicotine by either binding to an allosteric site or by interfering with a transduction mechanism. In the peripheral nervous system, the blockade of nicotinic cholinergic transmission at the ganglia by hexamethonium or mecamylamine and at the neuromuscular junction by decamethonium suggests that an ion channel may be associated with the nicotinic receptors in the ganglia that may be very similar to that found at the neuromuscular junction [36]. There are suggestions that blockade may result from the antagonist either acting directly with the channel or with the recognition site of the receptor [37]. Of course, it may be that mecamylamine can only interact with a specific conformation of the nicotine receptor, if it binds to it all.

The question arises as to what evidence there is for mecamylamine binding to a receptor other than the fact that it antagonizes nicotine. What are the structural requirements of the antagonistic properties of mecamylamine? What are the binding characteristics of the antagonists? Are the antagonistic properties of mecamylamine and pempidine confined only to nicotine?

Structure–activity relationship for nicotine antagonists

The structure–activity relationships of mecamylamine analogues in antagonizing nicotine-induced convulsions and pupil dilatation in mice were studied by Stone *et al.* [38]. Mecamylamine (*N*,2,3,3,-tetramethyl-2-norbornamine) possesses three chiral centers, making eight stereoisomers theoretically possible (Fig. 1). However, two of the chiral centers (carbons 1 and 4) are connected by a methylene bridge, limiting the possibilities to four. The isomers are divided into two groups, namely the exo- and endo-isomers. When the nitrogen extends away from the carbon cage, the compound is referred to as (±)-exo-mecamylamine. When the nitrogen is beneath the carbon cage, the compound is termed (±)-endo-

mecamylamine. Optical isomerism was found to have a minor role in the activities of these compounds in that the (+)-exo-isomer was similar in potency to the (±)-exo-isomer, as was racemic endo-mecamylamine. The N- and 2-methyl groups were found to be important in that increasing the chain length at either position significantly decreased activity. The N- and 2-demethylated forms of mecamylamine also had less activity than the parent compound. Furthermore, the methylene bridge, 3-gem-dimethyls, and 2-methyl groups were found to be important in that at least two of these three groups had to be present for optimal antagonistic activity. Also, translocation of the 2-methyl to the 1 position did not affect activity. The structural requirements for antagonism of nicotine-induced convulsions were correlated with ganglionic blockade ($r = 0.95$), suggesting a similar mechanism may be involved. These findings led Stone *et al.* [38] to conclude that mecamylamine has a specific action which probably involves a receptor.

Pempidine (*N*,2,2,6,6-pentamethylpiperidine), an optically inactive compound, has been shown to have mecamylamine-like properties (Fig. 1). Bretherick *et al.* [39] examined the activities of a number of pempidine derivatives in the cat nictitating membrane. The N-ethyl homologue of pempidine was found to be slightly more potent that the parent compound. It was found that the nature of the N-substitution was important for activity. Substitutions that decreased the base strength of the nitrogen resulted in greatly diminished activity, whereas electron-donating groups in this position enhanced the activity somewhat. Three of the four methyls in the 2- and 6-positions were required for activity; however, substitutions in the 4-position had no significant effect. The pyrollidine counterpart of pempidine (*N*,2,2,5,5-pentamethylpyrollidine) was found to be less potent than pempidine, and all four methyls in the 2- and 5-positions were necessary for activity. Double bonds in the 2:3 or 3:4 positions of pempidine, as well as in the 3:4 position of its pyrollidine counterpart did not alter activity significantly. A noncyclic analogue, di-*t*-butylamine, was found to be approximately equipotent with pempidine. These findings suggest that the base strength of the nitrogen and the substituents of the adjacent carbons are important for the pharmacological activity of pempidine.

These studies reveal structural requirements for nicotine antagonistic activity. However, these requirements are not as strict as those for many other classes of centrally acting drugs. The relatively low stereoselectivity is but one example.

Receptor binding of antagonists

As mentioned earlier, nicotine antagonists such as mecamylamine are unique in that they have not been used to study nicotine receptor binding *in vitro*. Earlier studies which were conducted using radiolabeled tubocurarine and decamethonium revealed low affinity binding to housefly brain [28]. Nordberg *et al.* [40] found high affinity binding to hippocampus in rat brain with [^{3}H]tubocurarine which suggested that there were C_{10} type receptors in brain. However, they did not evaluate other antagonists such as mecamylamine for binding to this site. Numerous studies have been conducted with the neurotoxins which have been reviewed earlier [35]. These data definitely show that the neurotoxins bind to mammalian brain tissue with high affinity. The significance of these binding sites remains in question. Despite similarities between nicotine and α-bungarotoxin binding sites, α-bungarotoxin is a weak competitor for [^{3}H]nicotine binding. Additionally, decamethonium and hexamethonium compete poorly for radiolabeled α-bungarotoxin binding.

Recent attempts in our laboratory to characterize an antagonist binding site using [^{3}H]pempidine have not been successful. Rat brain homogenates have been prepared and incubated with [^{3}H]pempidine under optimal conditions for [^{3}H]nicotine binding, and no saturable binding could be detected (unpublished observations). Attempts to optimize binding conditions for [^{3}H]pempidine have not provided more favorable results. These experiments have failed to provide evidence for a specific binding site for the reversible ganglionic blockers.

Molecular aspects of nicotinic acetylcholine receptors (nAChR)

The pharmacological features of the nAChR subtype have been characterized in fish electric organ and in the vertebrate peripheral nervous system, but not in the CNS. In addition, the regions of the molecule which form the two major functional domains—the ACh binding sites and the gated ion channel—have been defined. Is it possible that the features of these cholinergic receptors can provide an explanation for the antagonistic properties of mecamylamine and pempidine? This characterization has been due to the use of ligands such as ACh, nicotine and a number of snake venom toxins. Additional evidence has been derived from affinity labels that identify binding sites and more recently from monoclonal antibodies and cDNAs [41–43]. The structure of the nAChR derived from the neuromuscular junction and electroplax preparations is a closely packed rosette-like structure (Fig. 2) [42, 44–46] which is now known to be different biochemically and pharmacologically from those of the neuronal nAChR [41, 43, 47]. This transmembrane protein contains an ion-channel through the center of the oligomeric protein and forms the nAChR with which ACh combines to initiate end plate potentials [48, 49]. The nAChR of the neuromuscular junction consists of two α subunits and one each of β, γ, and δ, and the amino acid sequences of these subunits show a partial homology to those in the ganglia. However, the neuronal nAChRs purified from the brains of rats, cattle and chicken [50–52] consist of only α and β subunits with a putative stoichiometry of $\alpha_3\beta_2$ or $\alpha_2\beta_2$. These investigators have also demonstrated that in the human brain the nAChR binds nicotine with nanomolar affinity and have concluded that the neuronal nAChRs in the brain and ganglia have some similarities, but can be distinguished pharmacologically and immunologically.

Much progress in the biochemical and pharmacological characterization of the membrane constituents that participate in the transmembrane signalling process has been achieved. For example,

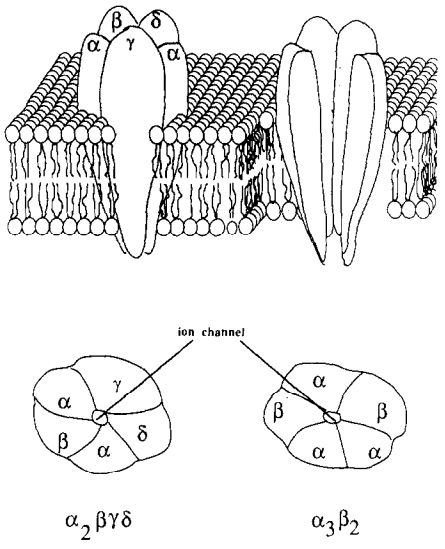

Fig. 2. Schematic models of the structure of the nicotinic acetylcholine receptor from the *Torpedo* electric organ (top panel) and the subunit composition of the neuromuscular nAChR arranged around the cation channel in a stoichiometric ratio of $\alpha_2\beta\gamma\delta$ (bottom panel). The ratio for the "putative" neuronal nAChRs is $\alpha_3\beta_2$.

evidence has been provided that the nAChR is a membrane bound allosteric protein with topologically distinct binding sites and mediates interactions between sites by characteristic transitions [41, 53]. In addition, the biochemical purification of these receptors has led to the cloning of the genes for all four receptor subunits from vertebrate skeletal muscle and the fish electric organ. Although the use of cDNAs and monoclonal antibodies as probes have revealed fundamental structural homologies between neuronal and neuromuscular nAChRs, a growing body of evidence suggests that brain nAChRs have amino acid sequences and probably subunit stoichiometry that differs considerably from those of the muscle and electric organ types (see Fig. 2) [51]. Furthermore, α-neurotoxins, such as α-bungarotoxin, bind to nAChRs with high affinity in the periphery and electric organ, but the distribution of the binding sites for [^{3}H]nicotine or [^{3}H]ACh (in the presence of a muscarinic antagonist and inhibition of AChE) contrasts markedly with the distribution of [^{125}I]-α-bungarotoxin binding sites in the brain [2]. Furthermore, the binding sites of [^{3}H]nicotine in the brains of rats, bovine and humans have relatively lower affinities for antagonists (e.g. mecamylamine, pempidine, hexamethonium) and higher affinities for cholinergic agonists [35, 43, 54]. It is therefore not surprising that studies involving the classical nicotinic antagonists such as mecamylamine, hexamethonium, and pempidine yield seemingly conflicting results between species and nAChR subtypes in both pharmacological and radioligand displacement studies.

Pharmacological properties of mecamylamine

It is possible that mecamylamine may produce its effects through multiple mechanisms, some of which may be noncholinergic. The evidence that the presynaptic localization of nAChRs in different brain regions modulates the release of other neurotransmitters [2, 55, 56] may, in part, cause the myriad effects on different systems which are known to be responsive to blockade by mecamylamine. The fact that antagonists such as mecamylamine and pempidine do not bind appreciably to brain structures, including the nicotine receptor, and the observations that antagonists such as α-bungarotoxin fail to block the excitation caused by ACh in various parts of the mammalian brain and in the spinal cord [23, 56–58] are reminders that the precise nature and function of the neuronal nAChRs and the molecular and cellular events leading to its activation and blockade are not well understood at the moment. Questions have even arisen as to whether or not ACh is the neurotransmitter at these sites [27, 59]. Evidence is also lacking to prove that these central binding sites are, in fact, functional nicotinic receptors. However, some compounds from a diverse chemical grouping, e.g. phencyclidine (PCP), chlorpromazine, histrionicotoxin and local anesthetics, are known to inhibit the cation channels of AChRs and thus block transmission through a site that interacts allosterically with the ACh binding site [53] with a reduction in the mean duration of channel opentime and/or facilitate the rate of desensitization [60]. More recently it was demonstrated that mecamylamine and pempidine blocked NMDA-stimulated release of NE by an action at the PCP site that is said to be linked to the NMDA ionophore complex—an action which does not involve nAChRs [61]. Other endogenous ligands may also play a role in the actions of mecamylamine. For example, it has been shown that substance P can alter the desensitization of the neuronal nAChR [62].

It should not be forgotten that antagonists such as mecamylamine may produce pharmacological effects of their own. For example, Aceto *et al.* [63] observed that, following chronic nicotine exposure to rats, mecamylamine did not prevent the body weight loss or block the suppression of drinking, but rather it increased water intake in its own right. Mecamylamine facilitates swimming endurance and shuttlebox performance in rats [64], impairs active avoidance learning in mice [65] and depresses shuttlebox behavior in guinea pigs [64], effects which may not necessarily be mediated directly through the cholinergic system. It is perhaps noteworthy that the blockade of convulsions in cats following intraventricular injection of nicotine is not selective to the nicotinic cholinoceptor antagonism by mecamylamine or hexamethonium alone, as reserpine which disrupts catecholaminergic, serotonergic and histaminergic mechanisms also suppresses the nicotine-induced convulsions in cats [66]. In other studies mecamylamine has been shown to prolong the cataleptic effect of morphine possibly by inhibiting dopamine release from striatal and mesolimbic dopaminergic neurons [67]. Studies in our own laboratories have shown that mecamylamine is very effective in lowering rectal temperature in mice (unpublished data).

Conclusions and future directions

Antagonists have played crucial roles in validating

most receptors in the central nervous system. In many cases, the antagonist rather than the agonist has been used directly to label the receptor. The fact that the functional roles of central nicotine binding sites have not been established may be due in large part to the inability of ganglionic antagonists to bind to central nicotine receptors. There could be many reasons for this apparent anomaly which could be either real or artifactual. One possibility may be that mecamylamine competes with nicotine for its receptor *in vivo*, but the conditions under which *in vitro* binding studies have been conducted are inappropriate for antagonist binding. There are suggestions that conformation of the receptor is critical for antagonist binding such as the hypothesis that nicotine opens an ion channel and the antagonist only binds to the "open" conformation of the receptor. Another possibility for the actions of mecamylamine is that mecamylamine and nicotine are binding to distinctly different sites in brain. There is some evidence that the actions of nicotine *in vivo* are mediated by two distinct receptor systems which are differentially sensitive to mecamylamine antagonism [68]. Of course, it is possible that mecamylamine could be exerting its antagonistic properties by altering a second messenger or through another neurotransmitter system that is integrated with the cholinergic system. Our knowledge of the transductive systems associated with the nicotine receptor is meager. The nature of the nicotine–mecamylamine interaction in the central nervous system must be better characterized. Thus, the search for mecamylamine analogs which may act at a specific central nicotinic site and lack ganglion blocking properties in the periphery may be valuable therapeutically and may serve as useful tools to characterize the nicotinic receptor subtypes. Also, the structural criterion for antagonistic activity has not been demonstrated unequivocally for the central effects of nicotine. The establishment of a specific binding site for mecamylamine would undoubtedly provide an important insight into the understanding of the central nicotine receptor.

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