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Potential Therapeutic Uses of Mecamylamine and its Stereoisomers

Justin R. Nickell^a, Vladimir P. Grinevich^{a,1}, Kiran B. Siripurapu¹, Andrew M. Smith^a, and Linda P. Dwoskin^a

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

Abstract

Mecamylamine (3-methylaminoisocamphane hydrochloride) is a nicotinic parasymphathetic ganglionic blocker, originally utilized as a therapeutic agent to treat hypertension. Mecamylamine administration produces several deleterious side-effects at therapeutically relevant doses. As such, mecamylamine's use as an antihypertensive agent was phased out, except in severe hypertension. Mecamylamine easily traverses the blood-brain barrier to reach the central nervous system (CNS), where it acts as a nicotinic acetylcholine receptor (nAChR) antagonist, inhibiting all known nAChR subtypes. Since nAChRs play a major role in numerous physiological and pathological processes, it is not surprising that mecamylamine has been evaluated for its potential therapeutic effects in a wide variety of CNS disorders, including addiction. Importantly, mecamylamine produces its therapeutic effects on the CNS at doses 3-fold lower than those used to treat hypertension, which diminishes the probability of peripheral side-effects. This review focuses on the pharmacological properties of mecamylamine, the differential effects of its stereoisomers, S(+)- and R(–)-mecamylamine, and the potential for effectiveness in treating CNS disorders, including nicotine and alcohol addiction, mood disorders, cognitive impairment and attention deficit hyperactivity disorder.

Keywords

mecamylamine; nicotinic acetylcholine receptors; smoking cessation; alcoholism; depression; anxiety

1.0 INTRODUCTION

The cholinergic neurotransmitter system plays a diverse role in numerous physiological processes of the central nervous system (CNS), including arousal, sleep, pain and cognitive function (Gotti and Clementi, 2004; Hogg et al., 2003), as well as in pathological conditions, including Alzheimer's disease, Parkinson's disease, depression and addiction (Newhouse et al., 1997; Picciotto and Zoli, 2008; Shytle et al., 2002e). Cholinergic signals are recognized and mediated by two pharmacologically distinct receptor classes: muscarinic and nicotinic

Corresponding Author: Justin Nickell, Ph.D., Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536, Tel: (859) 257-2555, Fax: (859) 257-7585, jnick2@uky.edu.

¹Current address: PET Center, Wake Forest Baptist Medical Center, Winston-Salem, NC 27157, United States

²Current address: Department of Psychology, University of Kentucky, Lexington, KY 40536, United States

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acetylcholine receptors (nAChRs). Muscarinic receptors mediate numerous central and peripheral nervous system functions (Eglen, 2005), the manipulation of which can lead to severe side-effects. As a consequence, effort has focused on the discovery of both novel agonists and antagonists as therapeutic agents which target nAChRs (Arneric et al., 2007; Bencherif and Schmitt, 2002; Dwoskin and Bardo, 2009; Dwoskin and Crooks, 2001; Gotti et al., 2006; Levin and Rezvani, 2000).

One of the first widely-used therapeutic agents targeting nAChRs was the noncompetitive antagonist mecamlamine (Banerjee et al., 1990; Martin et al., 1989). Mecamlamine was introduced originally by Merck & Co., Inc. as an antihypertensive agent (Stone et al., 1956). Although similar to the ganglionic blocker and quaternary ammonium compound hexamethonium, mecamlamine, a secondary amine, is unique among antihypertensive agents. Mecamlamine is rapidly and completely absorbed from the gastrointestinal tract, and has both a fast onset (37 min) and a relatively long duration of action (22 hr; Ford et al., 1956). Unfortunately, mecamlamine lacks nAChR subtype selectivity, and thus exerts antagonist activity at parasympathetic ganglionic receptors, which at therapeutic doses results in undesirable side-effects, including constipation, urinary retention and dry mouth and skin (Shytle et al., 2002a). However, unlike other ganglionic blockers, mecamlamine easily traverses the blood-brain barrier, allowing inhibition of nAChRs in the CNS (Martin et al., 1989; Suchocki et al., 1991). More recent studies report that central effects of mecamlamine are obtained at 3-fold lower doses than those used to treat hypertension (2.5–10 mg/day versus 30–90 mg/day), resulting in fewer and more manageable peripheral side-effects (Shytle et al., 2002a, d, e). Since nAChRs play a major role in numerous physiological and pathological processes, it is not surprising that mecamlamine has been evaluated as a potential therapeutic agent for a wide variety of disorders that affect the CNS.

Targacept, Inc., which secured the intellectual property, regulatory documentation, contracts and inventory related to mecamlamine from Layton Biosciences, Inc., received approval from the United States Food and Drug Administration (FDA) to test the effectiveness of mecamlamine in treating Tourette's syndrome, a neurological disorder characterized by involuntary movements (Sanberg et al., 2000). In 2002, Targacept, Inc. obtained use patents for the pure stereoisomers, S(+)- and R(–)-mecamlamine, for the treatment of a wide range of clinical conditions, including addiction, schizophrenia, hypertension and cancer (Shytle et al., 2002b, c). Additional use patents were secured for mecamlamine (racemic and stereoisomeric forms) to treat depression, bipolar disorder, obsessive compulsive disorder, attention deficit hyperactivity disorder (ADHD) and Alzheimer's disease (Sanberg et al., 2005). While to date the only FDA-approved use of mecamlamine is for "the management of moderately severe hypertension and uncomplicated cases of malignant hypertension", these patents have generated renewed excitement and investment in mecamlamine as a potential therapeutic for numerous CNS disorders.

In this review, we present an overview of the proposed therapeutic uses for mecamlamine, and both positive and negative results from preclinical and clinical studies. Also, results herein show an interaction between mecamlamine and nicotine, which likely will have an important impact on the development of mecamlamine in terms of doses employed and the conditions necessary to reveal efficacy. Future clinical studies should consider tobacco smoking status when selecting mecamlamine doses to be employed to obtain maximal efficacy for its proposed therapeutic uses.

2.0 NEUROPHARMACOLOGY

2.1 Mechanism of Action

Mecamylamine (3-ethylaminoisocamphane hydrochloride; Fig. 1, left) is a secondary amine that acts as a noncompetitive antagonist at all known nAChR subtypes (Connolly et al., 1992; Varanda et al., 1985). Interestingly, early studies reported that mecamylamine-induced inhibition of parasympathetic neurons from rat submandibular ganglia was the result of a competitive mechanism of action (Ascher et al., 1979; Gurney and Rang, 1984). However, mecamylamine inhibition of agonist-induced receptor activation could not be fully reversed, even after a 60-min washout, suggesting noncompetitive antagonism. In later studies utilizing cultures of parasympathetic neurons and medullary chromaffin cells, mecamylamine was shown to inhibit acetylcholine-evoked currents in a voltage-dependent manner, supporting a noncompetitive channel-blocking mechanism of action (Fieber and Adams, 1991; Nooney et al., 1992). Studies using cell expression systems, such as *Xenopus* oocytes expressing either rat or human nAChR subtypes, have demonstrated that mecamylamine inhibits both neuromuscular and neuronal nAChRs (Chavez-Noriega et al., 1997; Francis and Papke, 1996; Luetje and Patrick, 1991; Papke et al., 2008), providing additional evidence of its nonselective nAChR antagonist action.

The mechanism of action of mecamylamine in the CNS has been evaluated in our laboratory by determining its ability to inhibit nicotine-evoked [³H]dopamine (DA) release from superfused rat striatal slices (Fig. 2). Using a previously published method (Grinevich et al., 2003), rat striatal slices were incubated with 0.1 μM [³H]DA (final concentration; dihydroxyphenylethylamine, 3, 4-[7-³H]-; specific activity 28.0 Ci/mmol; Perkin Elmer Life and Analytical Sciences, Inc; Boston, MA) for 30 min and then superfused (1 ml/min) for 60 min with Krebs' buffer to establish a stable basal DA release rate. The superfusion buffer contained nomifensine (10 μM), a DA transporter inhibitor, and pargyline (10 μM), a monoamine oxidase inhibitor, to prevent reuptake and metabolism of DA, respectively, and to ensure that the [³H] collected in superfusate primarily represented parent neurotransmitter. Fractional release was calculated by dividing the amount of [³H] in each 5-min sample by the total tissue-[³H] at the time of sample collection. Basal [³H]DA outflow was calculated as the average fractional release in the two samples prior to addition of mecamylamine to the superfusion buffer. Total [³H]DA overflow was calculated by summing the increases in fractional release above basal release in response to nicotine in the absence and presence of mecamylamine.

Using these methods, the ability of mecamylamine to inhibit nicotine-evoked [³H]DA release from striatal slices was determined across a range of mecamylamine concentrations (10 nM–10 μM). Nicotine (10 μM) was added to the buffer 45 min after mecamylamine, and superfusion continued for an additional 65 min. Peak fractional [³H]DA release was observed 12 min following the addition of nicotine to the buffer, and [³H]DA levels gradually returned to basal levels despite the constant presence of nicotine (Fig. 2, top). Mecamylamine inhibited nicotine-evoked [³H]DA release in a concentration-dependent manner, with maximum inhibition achieved at 10 μM. A two-way repeated measures analysis of variance (ANOVA) with time and concentration as within-subject factors revealed main effects of time ($F_{15,330} = 24.0$, $p < 0.0001$) and concentration ($F_{4,22} = 1100$, $p < 0.0001$), and a time x concentration interaction ($F_{60,330} = 2.48$, $p < 0.0001$). Nonlinear regression revealed a sigmoidal function for mecamylamine inhibition with an $IC_{50} = 0.12 \pm 0.04$ μM and $I_{max} = 85 \pm 4\%$ (Fig. 2, bottom). Analysis of total [³H]DA overflow using a one-way ANOVA with concentration as a within-subject factor revealed a concentration-dependent effect of mecamylamine ($F_{4,12} = 24.1$, $p < 0.0001$). Furthermore, Schild analysis was performed to evaluate the mechanism by which mecamylamine inhibited nicotine-evoked [³H]DA overflow. Concentration-response for nicotine (0.1–100 μM) was

determined in the absence and presence of a single concentration of mecamylamine (1, 10, or 100 μM). For each mecamylamine concentration, the dose ratio (dr) was calculated as that producing an equivalent response to nicotine in the absence and presence of mecamylamine. The $\log(\text{dr}-1)$ was plotted as a function of \log mecamylamine concentration to provide the Schild regression. Results indicated that mecamylamine produced a rightward and downward shift of the nicotine concentration-response curve, which was not surmounted by increasing nicotine concentrations (Fig. 3). Further, linear regression generated by the Schild analysis (Fig. 3, inset) revealed a slope (slope = 0.07 ± 0.02 ; $r^2 = 0.94$) different from unity ($t_{28} = 29.1$; $p < 0.001$), consistent with noncompetitive inhibition (Arunlakshana and Schild, 1959; Kenakin and Boselli, 1989). These findings indicate that mecamylamine inhibits nicotine-evoked [^3H]DA release from striatal slices via a noncompetitive mechanism of action.

Others have shown that mecamylamine binds to a site within the nAChR channel pore, leading to a shortened duration of the open-channel state (Martin et al., 1990; Nelson and Lindstrom, 1999; Peng et al., 1994; Shen and Horn, 1998). However, a simple channel blocking mechanism is not sufficient to explain mecamylamine-induced inhibition. As such, a “trapping” mechanism for mecamylamine has been described (Lingle, 1983; Ostroumov et al., 2008), whereby mecamylamine binds to a site located deep within the channel pore, interfering with the ability of cations to permeate the channel, but not preventing the channel from closing once the agonist is removed. As a result, mecamylamine is trapped within the channel. In order for mecamylamine to exit the channel, the agonist must bind to the agonist recognition site in an orthosteric manner to produce a conformational change, returning the receptor to the open channel state (Gurney and Rang, 1984; Lingle, 1983; Skorinkin et al., 2004). A caveat of these studies is that they were performed in either non-neuronal (chromaffin cells) or neuromuscular models, and the interpretation of the results extrapolated to suggest a trapping mechanism for mecamylamine at nAChRs in the CNS.

Recent *in vitro* work from our laboratory determining [^3H]-mecamylamine binding to nAChRs expressed in rat brain membranes supports the hypothesis that mecamylamine is trapped within the pore of CNS nAChRs. Using a 20-point assay, saturation analysis of [^3H]-mecamylamine binding was performed to determine the maximum number of [^3H]-mecamylamine binding sites (B_{max}), affinity (K_d), and whether the ligand binds to one or more sites (Fig. 4). Rat brain membranes were prepared from whole brain and suspended in 4.8 ml of incubation buffer, providing a protein concentration of 140–160 μg in 100 μl . For the 20-point saturation assay, two stock solutions of [^3H]-mecamylamine (specific activities of 32.0 and 4.51 Ci/mmol; Perkin Elmer Life and Analytical Sciences, Inc; Boston, MA) were used to afford radioligand concentration ranges of 5–100 nM and 150–1500 nM, respectively. Samples were prepared by adding a single concentration of [^3H]-mecamylamine in 50 μl of buffer, 50 μl of 2.5 mg/ml bovine serum albumin (BSA), 100 μl of membrane suspension into polypropylene tubes, and 50 μl of buffer, for a total volume of 250 μl . Nonspecific binding was determined using 500 μM cold mecamylamine. Samples were incubated at room temperature for 30 min, and incubation stopped by addition of 3 ml of ice-cold incubation buffer followed by immediate filtration through S&S glass fiber filters (grade #32; presoaked overnight in 0.5% polyethylenimine) using a cell harvester. Filters were rinsed 3 times with 3 ml of ice-cold buffer and transferred to scintillation vials, 4 ml scintillation cocktail was added, and radioactivity determined by liquid scintillation spectroscopy. Protein was measured using the Bradford dye-binding procedure (Bradford, 1976), with BSA as the standard. [^3H]-Mecamylamine concentrations of up to 1500 nM were required to observe saturation (Fig. 4, top panel). Nonlinear regression revealed a K_d value of $1.27 \pm 0.18 \mu\text{M}$ and a B_{max} value of $2.92 \pm 0.93 \text{ pM/mg protein}$. Data did not fit a two-site model ($F_{2,56} = 0.1394$; $p = 0.87$). Scatchard transformation suggested the presence

of two binding sites (Fig. 4, top panel inset); however, nonlinear regression prohibited delineation of two linear representations of the Scatchard plot.

Effects of nicotine on [³H]-mecamylamine binding were determined. Binding assays were conducted using 100 nM [³H]-mecamylamine in the presence of a range of nicotine concentrations (0.01 nM–1.0 mM). Only the highest S(–)nicotine concentration (1 mM) significantly increased the amount of specific [³H]-mecamylamine binding from 117 ± 0.51 to 306 ± 39.7 fmol/mg protein ($p < 0.001$; not shown). Saturation analysis of [³H]-mecamylamine binding to rat brain membranes in the presence of 1 mM nicotine revealed ~2-fold increase in total [³H]-mecamylamine binding (Fig. 4, bottom panel). Insufficient saturation of total [³H]-mecamylamine binding precluded delineation of accurate values for B_{max} and K_d . However, the increase in [³H]-mecamylamine binding in the presence of nicotine provides evidence supporting binding within the nAChR channel, as channel opening increases in response to agonist. Thus, these findings support the hypothesis that mecamylamine binds within nAChR channels in the open conformational state, a prerequisite for trapping to occur.

2.2 Mecamylamine Stereoisomers

Over the past several decades, stereoselectivity has become an increasingly important consideration in the discovery and development of new therapeutic agents, largely due to improvements in stereoselective methods of synthesis and analyses (Jamali et al., 1989; Sastry, 1973; Smith, 1989; Waldeck, 2003), and to encouragement by the FDA with respect to new drug applications (De Camp, 1989). As a result, numerous racemic drugs, including mecamylamine, which previously received FDA approval, are being re-evaluated to determine potential benefits of the pure stereoisomers (Waldeck, 2003). Examination of the pharmacological properties of the mecamylamine stereoisomers (Fig. 1) began soon after the racemate was developed (Stone et al., 1962). Suchocki and colleagues (1991), using a mouse model, found no difference in potency between racemic mecamylamine, S(+)-mecamylamine or R(–)-mecamylamine with respect to inhibition of nicotine-induced decreases in spontaneous activity and antinociceptive effects. Similarly, no significant differences were observed between S(+)-mecamylamine and R(–)-mecamylamine inhibition of acetylcholine (ACh)-evoked currents in human $\alpha 3\beta 4$, $\alpha 4\beta 2$, $\alpha 3\beta 2$ and $\alpha 7nAChRs$ expressed in *Xenopus* oocytes, although S(+)-mecamylamine exhibited a significantly slower off-rate at $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs (Papke et al., 2001). Ability of the racemate, S(+)-mecamylamine and R(–)-mecamylamine (1 nM–1 mM) to inhibit [³H]-mecamylamine binding to rat whole brain membranes revealed no differences in affinity ($K_i = 1.53 \pm 0.33$ μ M, 2.92 ± 1.48 μ M and 2.61 ± 0.81 μ M, respectively; Fig. 5, top panel). Ability of nicotine (1 mM) to alter S-(+)- and R-(–)mecamylamine inhibition of [³H]-mecamylamine binding revealed no differences between the stereoisomers and the racemate (Fig. 5, bottom panel). Consistent with our previous findings, stereoisomer concentrations required to observe inhibition of [³H]-mecamylamine binding were 3-orders of magnitude higher in the presence than in the absence of nicotine (compare Fig. 5 top and bottom panels). With respect to clinical relevance, these results suggest that the mecamylamine stereoisomers may have lower efficacy in smokers compared with non-smokers, and that higher doses of mecamylamine stereoisomers may be required to observe therapeutic efficacy. Unfortunately, as the dose is increased, the likelihood of nonspecific side-effects is elevated. Therefore, it may be important for clinical trials investigating benefit of mecamylamine stereoisomers to take into account smoking status, as this may impact the study outcome.

In contrast to the previous research, stereoselective effects have been reported in other studies. S(+)-Mecamylamine was reported to inhibit low sensitivity $\alpha 4\beta 2$ nAChRs with better efficacy than R(–)-mecamylamine, while acting as a positive allosteric modulator of high sensitivity $\alpha 4\beta 2$ nAChRs (Fedorov et al., 2009). Low sensitivity $\alpha 4\beta 2$ nAChRs have a

stoichiometry of $(\alpha 4)_3(\beta 2)_2$, lower affinity for nicotine ($EC_{50} = 10 \mu M$) and are highly permeable to calcium ions, whereas high sensitivity $\alpha 4\beta 2$ nAChRs have a stoichiometry of $(\alpha 4)_2(\beta 2)_3$, greater affinity for nicotine ($EC_{50} = 1 \mu M$), and have low calcium permeability (Tapia et al., 2007). Positive allosteric modulation of high sensitivity $\alpha 4\beta 2$ nAChRs by S(+)-mecamylamine may be a contributing factor to the seemingly paradoxical procognitive effects of low-dose, racemic mecamylamine (see sections 3.8, 4.4), which is typically observed upon agonist activation of nAChRs. Interestingly, neuromuscular-type nAChRs appear to be more sensitive to R(-)-mecamylamine than to S(+)-mecamylamine (Papke et al., 2001), suggesting that S(+)-mecamylamine may be more effective at inhibiting neuronal nAChRs, and may be associated with a reduced side-effect profile.

3.0 PRECLINICAL BEHAVIORAL STUDIES

3.1 Nicotine Self-Administration

Nicotine, the major psychoactive compound in tobacco, is widely believed to play a primary role in maintaining smoking behavior (Rose, 2008). The use of mecamylamine in preclinical research has provided insights regarding the role of nAChRs in mediating the reinforcing properties of nicotine and the maintenance of smoking behavior (Katner et al., 2004; Lof et al., 2010; Newman et al., 2002; Palmatier et al., 2007; Phillips et al., 2007).

Numerous studies have shown that mecamylamine dose-dependently decreases intravenous nicotine self-administration (Le Foll and Goldberg, 2009, for review). Intravenous drug self-administration is the current gold-standard method to study the reinforcing properties of drugs. Self-administration involves implanting a catheter into the subject's jugular vein for delivery of drug following response on the active lever in the operant chamber. Drugs that are positively reinforcing (e.g., nicotine) increase the probability of future responses on the active lever. Using a fixed ratio schedule, wherein a fixed number of responses are required to receive a nicotine infusion, pretreatment with mecamylamine decreases responding for nicotine in rats, mice, dogs and nonhuman primates (DeNoble and Mele, 2006; Fowler and Kenny, 2011; Glick et al., 1996; Martin-Garcia et al., 2009; Nakahara, 2004; Palmatier et al., 2007; Risner and Goldberg, 1983; Shoaib et al., 1997; Spealman and Goldberg, 1982; Stolerman et al., 1999). Mecamylamine also decreases the breakpoint obtained using progressive ratio schedules of nicotine reinforcement (Mansbach et al., 2000). The progressive ratio schedule requires the subject to increase the number of responses to some designated value to obtain each subsequent drug infusion (i.e., reinforcer). Furthermore, the progressive ratio schedule is used to determine the relative reinforcement efficacy of a particular reinforcer and the maximum effort the subject will expend to obtain that reinforcer.

Methods used to assess potential for relapse to nicotine-seeking are cue-, stress- and/or nicotine-induced reinstatement. In the reinstatement assay, subjects self-administer drug to stability, after which extinction is instituted, in which responding no longer delivers the drug reinforcer. Over time, the subject reduces the number of responses on the active lever as extinction is established and a low rate of responding is maintained. To assess relapse of drug-seeking behavior, a stressor, the drug itself, or an environmental cue previously associated with the self-administered drug is reintroduced, which leads to resumption of responding on the active lever, however, the drug continues to be unavailable. If responding is increased above the extinction level, this is interpreted as reinstatement of drug-seeking behavior. Mecamylamine decreases cue-induced reinstatement of responding for nicotine in this model (Liu et al., 2006; Liu et al., 2007). Thus, mecamylamine inhibition of nAChRs effectively decreases both nicotine reinforcement and nicotine-seeking behavior.

3.2 Nicotine Drug Discrimination

Another behavioral paradigm frequently employed in the preclinical literature to assess the effects of nicotine is the drug discrimination assay (Morrison and Stephenson, 1969). In these studies, food- or water-deprived subjects are trained to press the lever to obtain food or water. Once the operant response has been established, drug discrimination training ensues. Nicotine is injected prior to the session and responses on one (active) of the two levers in the operant chamber results in reinforcement, whereas responses on the other lever (inactive) has no programmed consequence. On alternating days, saline is injected prior to the session, and responses on the other lever result in reinforcement. Once the subject meets the training criteria, drug discrimination is evaluated. A test drug is injected prior to the session, and discrimination of the test drug as nicotine-like is indicated by the subject responding on the nicotine-associated rather than the saline-associated lever. To determine if the test drug inhibits the discriminative stimulus effects of nicotine, the test drug is administered prior to nicotine. If responding on the lever associated with nicotine is reduced, the results are interpreted as inhibition of the discriminative stimulus effects of nicotine. Pretreatment with racemic mecamylamine consistently blocked the discriminative effects of nicotine, which was not overcome by increasing the nicotine dose (Stolerman et al., 1983). Numerous studies have replicated these results (Brioni et al., 1994; James et al., 1994; Jutkiewicz et al., 2011; Olausson et al., 2004; Zaniwska et al., 2006). Thus, mecamylamine blockade of the discriminative stimulus effects of nicotine provides support for the involvement of nAChRs in this response to nicotine.

3.3 Nicotine-Mediated Classical Conditioning

Racemic mecamylamine also has been shown to block classical conditioning produced by nicotine. Classical conditioning involves the pairing of a conditioned stimulus with an unconditioned stimulus, which generates a specific response. Following a number of pairings, the conditioned stimulus generates the same response as the unconditioned stimulus. Thus, the response becomes a conditioned response and occurs in the absence of the unconditioned stimulus. A common behavioral task employing the classical conditioning approach is the conditioned place preference assay, which assesses the secondary, conditioned reward induced by a drug (Rossi and Reid, 1976). This assay is thought to assess DA-mediated incentive learning and to measure the rewarding effects of drugs (Bardo and Bevins, 2000; Di Chiara, 2000).

In the context of the current review, nicotine-induced conditioned place preference provides a measure of the secondary conditioned reward produced by nicotine. Subjects are typically initially allowed free access to the entire two- or three-chamber apparatus. Subsequently, nicotine is administered to the subject, and then the subject's access is restricted to one of the environmentally-distinct chambers within the apparatus. On alternate sessions, saline is administered and the subject restricted to the other distinct chamber within the apparatus. On the test session, the subject has free access to the entire apparatus, and a greater amount of time spent in the chamber that was paired with nicotine indicates conditioned place preference has been established. Nicotine has been shown to produce conditioned place preference in rats, mice and non-human primates (see Le Foll and Goldberg, 2009 for review). Pretreatment with mecamylamine blocks the nicotine place preference (Fudala et al., 1985; Grabus et al., 2006; Iwamoto, 1990; Sershen et al., 2009), indicating that activation of nAChRs mediates the rewarding properties of nicotine.

Furthermore, mecamylamine has been shown to attenuate the reinstatement of nicotine conditioned place preference (Biala et al., 2010), which suggests that mecamylamine may have efficacy in attenuating nicotine relapse. In this assay, nicotine conditioned place preference is extinguished by allowing animals free access to the entire apparatus for several

trials in the absence of drug pairing. Following extinction, the animals are primed with a small dose of nicotine, which reinstates the preference for the chamber formerly paired with nicotine. Importantly, in contrast with muscarinic acetylcholine receptors antagonists (e.g., atropine and scopolamine), mecamylamine does not produce conditioned place avoidance, i.e., mecamylamine alone does not decrease the amount of time spent in the mecamylamine-paired environment (Zarrindast et al., 2003). Thus, mecamylamine blocks the rewarding effects of nicotine, and this is not due to intrinsic aversive properties of mecamylamine.

Another behavioral task commonly used to measure the secondary conditioned effects of nicotine is conditioned taste avoidance (Garcia, 1985). In the conditioned taste avoidance assay, a substance with a benign or pleasant taste (e.g., saccharin; conditioned stimulus) is paired with a substance that causes gastric malaise (e.g., radiation; unconditioned stimulus). With pairings of the conditioned and unconditioned stimuli, the conditioned stimulus elicits the conditioned response. Nicotine, when paired with a saccharin solution, produced conditioned taste avoidance, and pretreatment with mecamylamine blocks the conditioned taste avoidance (Kumar et al., 1983). These initial results have been confirmed by others (Bevins et al., 2006; Iwamoto and Williamson, 1984; Rauhut et al., 2008), implicating nAChRs in mediating nicotine-induced conditioned avoidance. However, an alternative interpretation is that the decrease in consumption of saccharin-solution following nicotine administration is not conditioned taste avoidance, but an example of anticipatory contrast. The suppression of a conditioned stimulus (e.g., sweet taste of saccharin) is suggested to occur because the relative value of the reward from the conditioned stimulus is irrelevant compared with the reward from the highly preferred psychoactive drug (e.g., nicotine) that directly activates dopaminergic pathways in the brain (Grigson, 1997). Nicotine reward is known to be dopamine-dependent (Di Chiara et al., 2004). Thus, the decreased intake of saccharin-solution may be due to the rewarding effects of nicotine, rendering the reward obtained from saccharin irrelevant. Therefore, mecamylamine blockade of the rewarding properties of nicotine may be preventing formation of conditioned taste aversion.

Interestingly, mecamylamine also decreases conditioned place preference induced by morphine. Microinjection of mecamylamine (1 – 7.5 μg) into the ventral tegmental area and basolateral amygdala of rats dose-dependently decreases morphine-induced conditioned place preference (Rezayof et al., 2007; Zarrindast et al., 2005). nAChRs in the CA1 region of the dorsal hippocampus have been shown to play a role in morphine-induced conditioned place preference (Rezayof et al., 2006). Microinjections of nicotine (0.5, 0.75 and 1 μg) into the CA1 region potentiated the effect of an ineffective systemic morphine dose (0.5 mg/kg), and elicited conditioned place preference. Bilateral injection of mecamylamine (2, 4 and 8 μg) into the CA1 region decreased both morphine-induced conditioned place preference and the nicotine potentiation of the morphine response. These results indicate that morphine reward is at least partially mediated by nAChRs.

Together, this research shows that mecamylamine inhibits the reinforcing and rewarding effects of nicotine. Since mecamylamine acts as an antagonist at all nAChR subtypes, the specific nAChR subtypes involved have not been elucidated by this research. Studies are currently aimed at identifying specific nAChRs involved in the behavioral effects of nicotine using knockout mice, subtype-selective neurotoxic peptides and small molecules (Brunzell, 2012; Cahir et al. 2011; Exley et al., 2011; Neugebauer et al., 2006; Picciotto et al., 2001; Wooters et al., 2011). Discussion of this work is beyond the scope of the current review; however, results from these investigations may further define new pharmacological targets and augment the discovery of novel therapeutics as smoking cessation agents.

3.4 Alcohol Abuse

Alcohol and nicotine are the most commonly co-abused drugs (Ait-Daoud et al., 2005; Littleton et al., 2007; Meyerhoff et al., 2006). A growing body of literature suggests that nicotine and alcohol co-abuse is due to modulation of nAChR expression and function (Collins, 1990; Davis and de Fiebre, 2006; Dohrman and Reiter, 2003; Funk et al., 2006; Larsson and Engel, 2004). Mecamylamine decreases behaviors resulting from either acute or chronic alcohol administration. Hyperactivity produced by alcohol is decreased by mecamylamine at doses (1–6 mg/kg) that do not alter locomotor activity (Blomqvist et al., 1992; Larsson et al., 2002). Furthermore, mecamylamine decreased ethanol-induced psychomotor sensitization, without altering locomotor activity or blood alcohol levels (Bhutada et al., 2010). Mecamylamine prevented formation of ethanol conditioned place preference following restraint and forced swim stress (Bhutada et al., 2012), indicating that mecamylamine may be efficacious in reducing alcohol relapse following introduction to a stressful environment. Mecamylamine also decreased the locomotor response to alcohol in FAST mice (Kamens and Phillips, 2008), a selectively bred line with increased locomotor sensitivity to acute alcohol (Phillips, 1993). Interestingly, mecamylamine had no effect on hyperactivity induced by acute exposure to cocaine or methamphetamine in FAST mice (Kamens and Phillips, 2008), suggesting that nAChRs specifically mediate the response to alcohol in these animals.

Systemic administration of mecamylamine decreases voluntary alcohol intake in rats and mice using both a simple two bottle choice test and operant self-administration (Farook et al., 2009; Ford et al., 2009; Kuzmin et al., 2009; Le et al., 2000). Also, mecamylamine decreases alcohol intake in high alcohol-preferring C57Bl/6J mice using the “drinking in the dark” method, in which access to 20% ethanol for 2 or 4 hr during the dark cycle produces relevant blood alcohol concentrations (Hendrickson et al., 2009; Rhodes et al., 2005). Microinjections of mecamylamine into the ventral tegmental area and nucleus accumbens, two regions that play a critical role in addiction (Self, 2004; Vetulani, 2001), decreased alcohol self-administration in rats (Ericson et al., 1998; Löf et al., 2007; Nadal et al., 1998). Moreover, a recent microdialysis study demonstrated that microinjection of mecamylamine (100 μ M) into anterior, but not posterior, ventral tegmental area is sufficient to decrease alcohol-evoked DA release *in vivo* (Ericson et al., 2008). Collectively, these studies suggest that nAChRs in ventral tegmental area and nucleus accumbens play an important role in the reinforcing effects of alcohol. Moreover, these results provide evidence for the use of mecamylamine as a therapeutic for alcohol addiction. However, the studies are limited because the effects of mecamylamine were not determined in a model in which animals are experienced with both nicotine and alcohol, as in the human condition. It may be that nAChR antagonists, such as mecamylamine, exhibit greater therapeutic benefit in the presence of nicotine, although higher doses of mecamylamine may be required. Future studies investigating effects of acute and chronic administration of alcohol plus nicotine are needed.

3.5 Methamphetamine Abuse

Nicotine and methamphetamine abuse appear to be linked (Goldsamt et al., 2005). Preclinical studies have begun to investigate interactions between these abused drugs. With respect to the locomotor stimulant effects of methamphetamine, mecamylamine did not alter methamphetamine-hyperactivity in FAST mice (Kamens and Phillips, 2008), suggesting that nAChRs play a limited role in this effect of methamphetamine. However, nicotine and methamphetamine substitute for one another in the drug discrimination assay (Gatch et al., 2008). Mecamylamine decreases the discriminative stimulus effects of methamphetamine in rats (Desai and Bergman, 2010). Furthermore, mecamylamine blocks the ability of nicotine to substitute for methamphetamine (Gatch et al., 2008). Thus, although locomotor stimulant

effects of methamphetamine do not appear to involve nAChRs, nAChR activation underlies the interoceptive cues elicited by methamphetamine.

Mecamylamine also decreases methamphetamine self-administration in rats following local injection into the medial habenula or interpeduncular nucleus (Glick et al., 2008). This effect of mecamylamine was suggested to be the result of inhibition of $\alpha 3\beta 4^*$ nAChRs, since this nAChR subtype is found in high density in these brain regions (Klink et al., 2001; Quick et al., 1999). When administered systemically, nicotine attenuated cue-induced and methamphetamine-induced reinstatement of methamphetamine-seeking in rats, although mecamylamine did not induce reinstatement (Hiranita et al, 2006). One interpretation of these findings is that nicotine decreases reinstatement of methamphetamine-seeking via activation of nAChRs, rather than by receptor desensitization. Collectively, these data demonstrate that nAChRs may be viable targets for the development of pharmacotherapeutic agents to treat methamphetamine abuse.

3.6 Cognition

Hippocampus and frontal cortex mediate mammalian cognitive processes and receive rich cholinergic input from the medial septal nucleus and nucleus basalis of Meynert. Literature has accumulated focusing on the influence of nicotine and nAChR antagonists on learning and memory. Many of these investigations employed the radial-arm maze to assess spatial and non-spatial learning and memory in rodents (Olton and Samuelson, 1976). The radial-arm maze consists of a central hub, from which a number of arms radiate. A food reward, which is not detectable from the central hub, is placed at the termini of a subset of arms. In the absence and presence of a test drug, the rodent is allowed access to the arms for a fixed duration of time with the goal of locating those arms which contain food. During successive trials, the animal returns to those arms which were previously baited. In a slightly modified version of the assay, the alternative arms are baited, and the animal must relearn the location of the food. Entry of arms lacking the food reward is considered an error, i.e., a reduction in choice accuracy. A preponderance of data demonstrate that nicotine enhances choice accuracy, while nAChR antagonists, including mecamylamine, impair choice accuracy, resulting in a greater number of errors (Levin et al., 1987, 1989, 1997; Levin and Rose, 1990; McGurk et al., 1991). Of importance, mecamylamine-induced inhibition of cognitive processes is dose-dependent. While high doses of mecamylamine negatively altered memory, low doses (< 1 mg/kg) enhanced memory (Levin et al., 1993; Levin and Caldwell, 2006). Corroborating evidence regarding the dose-related effects of mecamylamine on memory has been obtained in aged non-human primates, such that low doses of mecamylamine improved memory in the delayed matching to sample task (Terry et al., 1999).

Differential effects of mecamylamine on memory across a range of doses may be due to a greater sensitivity of specific nAChR subtypes to mecamylamine. Although mecamylamine is considered a nonselective nAChR antagonist, its inhibitory efficacy at $\alpha 2\beta 4$ and $\alpha 4\beta 4$ nAChRs is greater than at $\alpha 2\beta 2$, $\alpha 4\beta 2$ and $\alpha 7$ nAChRs (Chavez-Noriega et al., 1997). nAChR subtype heterogeneity occurs throughout the brain and local application of nAChR antagonists results in brain region-dependent alterations in memory demonstrated using the radial arm maze task (Levin et al., 2006). Another explanation for differential effects of mecamylamine doses on memory is that low doses of mecamylamine may produce effects similar to those following desensitization of nAChRs (Collins et al., 1988; Damaj et al., 1996; James et al., 1994). Thus, specific mechanisms underlying the differential dose-related effects of mecamylamine on memory and cognition have not been fully elucidated.

Similar to the improvements in cognition found following administration of low doses of mecamylamine, cognitive processes have been shown to be enhanced following low doses

of methyllycaconitine, an $\alpha 7$ -selective nAChR subtype antagonist (Hahn et al., 2011). Rats were trained to perform a 5-choice serial reaction time task, in which a light stimulus was randomly presented in one of several holes, and a nose poke in the illuminated hole either while illuminated or within five seconds of the light being extinguished resulted in delivery of a food pellet reward. An incorrect response was recorded when the rat nose-poked into any other hole, and a failure to issue a response was recorded as an omission error. Following stable responding, rats were systemically administered nicotine or vehicle in the presence of increasing doses of methyllycaconitine. As expected, nicotine reduced the number of omission errors, and this effect was reversed by methyllycaconitine in a dose-dependent manner. In the absence of nicotine, however, low doses of methyllycaconitine (0.4 and 1.3 mg/kg) improved response accuracy. In contrast, dihydro- β -erythroidine, a competitive antagonist at $\alpha 4\beta 2$, $\alpha 4\beta 4$, $\alpha 3\beta 2$ and $\alpha 2\beta 2$ nAChRs, neither increased the number of correct responses nor reduced the number of omission errors. Together, these results suggest that low doses of nAChR antagonists augment cognition and that the $\alpha 7$ nAChR subtype may serve a primary role in modulating facets of attentional awareness mediating response accuracy.

Another potential mechanism underlying the procognitive effects of low doses of mecamylamine may be activation of downstream signaling events resulting from nAChR antagonism. Alzheimer's disease is characterized by impairments in cognition associated with a loss of basal forebrain cholinergic neurons (Etienne et al., 1986; Lehericy et al., 1993; Whitehouse et al., 1981), and a marked reduction in levels of nerve growth factor and its target receptor, TrkA (Boissiere et al., 1997; Mufson et al., 1997). Basal forebrain cholinergic neurons are highly dependent upon continuous exposure to nerve growth factor for proper maintenance and survival, and nicotine increases nerve growth factor levels in cortex, hippocampus and striatum (Bellurdo et al., 1998; French et al., 1999). Exposure of PC12 cells to nicotine (0.01–10 μ M for 24 hr) resulted in increased TrkA expression and this effect was reversed by simultaneous exposure to mecamylamine (5 μ M for 24 hr; Jonnala et al., 2002). Importantly, exposure to low concentrations of mecamylamine (10 and 100 nM) alone resulted in a marked elevation in TrkA receptor expression compared to control. To evaluate the effect of mecamylamine on TrkA receptor expression *in vivo*, rats were administered mecamylamine (24 mg/kg/day) via an indwelling catheter, and a modest elevation (20%) in TrkA receptor expression was found (Jonnala et al., 2002). Collectively, these studies provide some evidence supporting the idea that stimulation of downstream targets may underlie the positive effects of low doses of mecamylamine on cognition.

Thus, low doses of mecamylamine may provide cognitive benefits. However, when used therapeutically, the dose of mecamylamine should be considered carefully, since, as with other therapeutics, the beneficial effects of high doses must be balanced by potential negative side-effects, including negative effects on cognition. Additionally, consideration of the patient's tobacco smoking status is important, as some of the negative effects on cognition induced by mecamylamine may be surmounted by nicotine (Levin et al., 1997b). Thus, the efficacy of mecamylamine as a therapeutic may be compromised by nicotine due to concurrent tobacco smoking.

3.7 Chronic Mecamylamine

A preponderance of studies evaluating effects of mecamylamine have investigated effects of acute administration, whereas few studies have evaluated effects of chronic mecamylamine administration. Continuous mecamylamine treatment has been reported to result in brain region- and subtype-specific upregulation of nicotinic receptors (Abdulla et al., 1996; Collins et al., 1994; Pauly et al., 1996; Peng et al., 1994). With respect to behavioral analyses, one study reported that similar to acute mecamylamine administration, chronic treatment of rats with mecamylamine (3 mg/kg/day) eliminated the improvement in working

memory resulting from acute nicotine administration (Levin et al., 1993). Importantly, when mecamylamine alone was administered chronically, working memory was increased during the first week of treatment, however, tolerance developed to this effect in subsequent weeks of treatment. Further work revealed that chronic mecamylamine briefly increased choice accuracy, a facet of cognitive function, but with continued administration choice accuracy was hindered (Levin et al., 1997a). Transient improvements in working memory and choice accuracy may be prolonged with continuous administration of low-doses (< 1 mg/kg) of mecamylamine; however studies evaluating low doses have not been reported in the literature.

3.8 Antidepressant and Anxiolytic

While it is not possible to develop an animal model that measures “depression” per se (Cryan and Mombereau, 2004), several preclinical behavioral paradigms are used typically to identify antidepressant-like effects of drugs. Perhaps the most widely used assay is the forced swim test, in which a rodent is placed in a water-filled cylinder from which it cannot escape, and total distance travelled and length of time required for immobilization (i.e., cessation of escape-oriented movements) are measured (Porsolt et al., 1977). Administration of an antidepressant drug prior to the forced swim test increases both distance travelled and length of time until immobilization is reached (Cryan et al., 2005). Another behavioral assay used to measure antidepressant-like effects is the tail suspension test, wherein the animal (predominantly mice) is suspended in mid-air by the tail, and the length of immobilization time is measured. Similar to the results in the forced swim test, antidepressants decrease the length of time during which the animal is immobile (Caldarone et al., 2004; Holmes et al., 2002).

Importantly, preclinical research has demonstrated that many currently available antidepressants, including bupropion, reboxetine, fluoxetine, sertraline, desipramine, paroxetine, nefazodone and venlafaxine act as noncompetitive antagonists at nAChRs (Fryer and Lukas, 1999; Miller et al., 2002a, b). Thus, it is not surprising that mecamylamine (1–10 mg/kg) administered acutely increased distance travelled in the forced swim test and decreased the length of time immobilized in the tail suspension test (Andreasen et al., 2009; Caldarone et al., 2004). Mecamylamine decreased distance travelled in the open-field assay across the same dose range that increased distance travelled in the forced swim test, suggesting that these effects of mecamylamine are not due to nonspecific stimulant effects (Andreasen et al., 2009). Studies employing $\beta 2$ or $\alpha 7$ nAChR subunit knockout mice found that while mecamylamine increased the time to immobilization for wild-type mice in the forced swim test, the $\beta 2$ and $\alpha 7$ knockout mice were insensitive to this effect of mecamylamine. These results suggest that nAChRs containing $\beta 2$ or $\alpha 7$ subunits may mediate the antidepressant-like effects of mecamylamine (Caldarone et al., 2004; Rabenstein et al., 2006). Further, mecamylamine potentiates the antidepressant-like effects of the selective serotonin reuptake inhibitor (SSRI), citalopram, and the tricyclic antidepressant, imipramine, in the tail suspension test (Popik et al., 2003). An interpretation of these results is that mecamylamine may potentiate the behavioral effects of these antidepressants by blocking serotonin reuptake (Ma et al., 2006). This mecamylamine-induced potentiation of the effects of citalopram was not replicated in a subsequent study; further, potentiation of the antidepressant-like effects of reboxetine, a selective norepinephrine reuptake inhibitor, were not observed in either the tail suspension test or forced swim test (Andreasen and Redrobe, 2009a). Thus, mecamylamine may have potential as an adjunct therapy for depression, however, discrepant results from the preclinical literature cast some doubt on this therapeutic application for mecamylamine.

Importantly, several preclinical studies have shown that repeated administration of nicotine produces antidepressant-like effects similar to those observed following acute

mecamylamine administration (Djuric et al., 1999; Ferguson et al., 2000). This apparent contradiction, that a nAChR agonist and nAChR antagonist produce the same pharmacological effect, may be explained by agonist-induced receptor desensitization leading to functional antagonism of the receptor (Buccafusco et al., 2009; Gentry and Lukas, 2002; Giniatullin et al., 2005; Picciotto et al., 2008; Quick and Lester, 2002; Reitstetter et al., 1999). Classical work by Katz and Thesleff (1957) led to the concept of nAChR desensitization. Upon binding of nicotine to its recognition sites on the receptor, a rapid conformational change in the protein occurs; the ion channel opens allowing cation flux across the membrane, and the receptor shifts to a high-affinity desensitized state. Thus, upon initial receptor binding, nicotine acts as an agonist eliciting a cellular response (channel opening). Also, nicotine acts as a functional antagonist, inducing receptor desensitization and rendering the receptor unresponsive to subsequent exposure to agonist. Thus, nAChR antagonists such as mecamylamine mimic the desensitization produced by nicotine by prohibiting channel function, without providing an antecedent agonist-like response.

Observations that nAChR agonists and nAChR antagonists produce the same pharmacological effect are further complicated by studies showing that concentrations of agonist required to activate the receptors are significantly greater those which desensitize the receptor (Frazier et al., 2003; Grady et al., 1994). Thus, agonists, depending on the concentrations, can produce outcomes either similar or in opposition to the effect of antagonists in the nAChR system. With respect to the current topic, both agonist (e.g., low concentrations of nicotine or repeated administration) and antagonists (e.g., mecamylamine) produce antidepressant effects.

The role of nAChR desensitization in the antidepressant-like effects of nicotine is supported also by findings that acute mecamylamine reverses these antidepressant-like effects of nicotine in the Flinders Sensitive Line (FSL) of rats (Tizabi et al., 2000), another animal model of depression (Overstreet, 1993; Overstreet et al., 1995). However, results in the forced swim test appear to be dependent, at least in part, on the subject's genetic background. Acute mecamylamine, but not nicotine, increased swim distance in inbred NMRI mice, whereas nicotine, but not mecamylamine, increased swim distance in outbred C57BL/6J mice, and both drugs increased swim distance in inbred BALB/c mice (Andreasen and Redrobe, 2009b). These results support previous observations that baseline activity exhibited by these strains covaries with drug responsiveness in this assay (Petit-Demouliere et al., 2005). Nonetheless, these findings indicate that the efficacy of mecamylamine as an antidepressant may in part depend upon prior history of nicotine use, as well as the genetic background of the individual.

The interaction of mecamylamine with targets downstream from AChRs also may contribute to its antidepressant effects. Depression has been correlated with the expression of brain-derived neurotrophic factor (BDNF), a protein supporting the survival of neurons and facilitating the differentiation and growth of new neurons. Specifically, clinical depression has been reported to be associated with low serum BDNF levels (Karege et al., 2002; Molendijk et al., 2011). Also, suicide victims prone to bouts of major depression have been shown to have reduced BDNF levels in both cerebral cortex and hippocampus (Dwivedi et al., 2003). Preclinical studies show that mice which were genetically modified to express low levels of BDNF display an increase in anxiety- and depression-like behaviors (Martinowich et al., 2007). Furthermore, several antidepressants, including fluoxetine and citalopram, elevate BDNF levels (Molteni et al., 2006; Russo-Neustadt et al., 2004), in addition to acting as nAChR antagonists (Fryer and Lukas, 1999).

Based on the above reports, one could hypothesize that the antidepressant-like properties of mecamylamine may be the result of elevations in BDNF expression. Several lines of

evidence support this hypothesis. Using cell culture, differentiated SH-SY5Y cells treated with mecamylamine (0.01–10 μM) exhibited significantly increased BDNF transcript levels (Park et al., 2011). Results also showed that systemic administration to rats of a single low dose (0.3 mg/kg) of mecamylamine elevated cerebral cortical BDNF expression 200% relative to control (Park et al., 2011). Further, to assess the behavioral response to mecamylamine and the relationship to BDNF levels, this group of investigators employed the elevated plus maze. The elevated plus maze consists of two completely enclosed arms and two open exposed arms; the duration of time spent in the open and closed arms is quantified. Animals with low levels of anxiety spend more time in the open arms than in the closed arms. Results show that mecamylamine produced an anxiolytic effect, reducing the dexamethasone-induced increase in the amount of time spent in the closed arm of the elevated plus maze. Moreover, the observed anxiolytic effect of mecamylamine correlated with an increase in cortical BDNF levels obtained immediately following the behavioral test (Park et al., 2011). Thus, beneficial pharmacological effects of mecamylamine may be, in part, the result of effects on targets downstream from nAChRs, such as changes in BDNF expression.

3.9 Mecamylamine Stereoisomers and Behavior

Consistent with the majority of research performed using racemic mecamylamine, several preclinical studies have reported that each of the stereoisomeric forms of mecamylamine have antidepressant-like effects. For example, S-(+)mecamylamine increased the total distance travelled by both mice and rats in the forced swim test (Lippiello et al., 2008). S-(+)Mecamylamine also decreased anxiety-like behavior in rats, as evidenced by an increase in social interaction and an increase in percentage of time spent in the light side of the light/dark box, two assays revealing anxiety-like behavior in rodents (Lippiello et al., 2008). Conversely, R-(–)mecamylamine was anxiolytic in the forced swim test when evaluated using several strains of mice; however, this effect was not observed in all strains (Lippiello et al., 2008). Further, R-(–)mecamylamine was less potent (30-fold) than S-(+)mecamylamine in the forced swim and social interaction tests, and exhibited no activity in the light/dark box (Lippiello et al., 2008). Similarly, the S-(+)mecamylamine was more effective at blocking nicotine-induced seizures than R-(–)mecamylamine (Newman et al., 2001). Moreover, while S-(+)mecamylamine and R-(–)mecamylamine appear to have a similar potency in blocking nicotine-induced antinociception, only S-(+)mecamylamine decreased spontaneous locomotor activity (Suchocki et al., 1991). Taken together with results from cell expression systems that indicate S-(+)mecamylamine is more selective for neuronal nAChRs than R-(–)mecamylamine (Papke et al., 2001), S-(+)mecamylamine may better target nAChRs associated with anxiolytic/antidepressant-like effects and have the advantage of fewer peripheral side effects.

4.0 CLINICAL STUDIES

4.1 Smoking Cessation

Given the preclinical evidence demonstrating that mecamylamine decreases the reinforcing and rewarding effects of nicotine, it is not surprising that clinical studies have investigated the potential for mecamylamine to act as a smoking cessation agent. Early studies evaluated the ability of mecamylamine to treat nicotine dependence (Tennant et al., 1984; Tennant and Tarver, 1984). A group ($n = 14$) of nicotine-dependent subjects was given 5–10 mg mecamylamine daily for 21 days and the dose was escalated until patients either experienced a decrease in nicotine craving or until toxic effects were observed (Tennant et al., 1984). Results showed that 13 of 14 subjects (93%) had a reduction in nicotine craving and 7 subjects (50%) ceased smoking completely. In another study, subjects were administered 20 mg mecamylamine daily for 6 weeks or 50 mg/day for 3 weeks (Tennant and Tarver, 1984).

Results showed that the higher mecamylamine dose reduced nicotine intake as measured by self-report and urine analysis, but that side-effects, including constipation, urinary retention, abdominal cramps and weakness, were pronounced. The lower dose (20 mg/day) of mecamylamine was less effective, but was better tolerated. Thus, while mecamylamine may be a viable treatment for some cases of recalcitrant nicotine dependence, a high prevalence of side-effects diminished its therapeutic value. In contrast, several studies reported that mecamylamine transiently increases tobacco consumption and nicotine intake (Pomerleau et al., 1987; Rose et al., 1998), consistent with the preclinical literature (Glick et al., 1996). The mecamylamine-induced increase in tobacco consumption was thought to be the result of an “extinction burst” and an attempt to overcome the consequent reduction in nicotine reward (Rose et al., 1998).

The side-effect profile of mecamylamine, together with the observed increased tobacco consumption, led to a lack of interest in this drug as a smoking cessation agent and treatment for nicotine dependence. However, interest in mecamylamine renewed in testing the hypothesis that a combination agonist-antagonist treatment may be effective as a smoking cessation therapy (Rose and Levin, 1991; Rose et al., 1994). The idea was that an antagonist would block the reinforcing effects of nicotine, while an agonist would minimize withdrawal symptoms. Nicotine replacement therapy (NRT; 21 mg/day) was given transdermally either alone or in combination with mecamylamine (5 mg oral tablet, twice daily for 2 weeks) to nicotine-dependent patients. Mecamylamine relieved craving and alterations in affect following the onset of smoking cessation. The low dose of mecamylamine resulted in minimal side-effects, including constipation, dry mouth and orthostatic hypotension. In another study by this group of investigators (Rose et al., 1998), the effectiveness of mecamylamine (10 mg/day) plus nicotine (21 mg/day) for 4 weeks prior to the initiation of smoking cessation resulted in a higher percentage of abstinent subjects compared with those that did not receive mecamylamine (47.5 vs. 27.5%). Also, the combined treatment of mecamylamine plus nicotine reduced smoking satisfaction and craving when compared with administration of either drug alone. Furthermore, continuous abstinence at 6 months was higher (40%) in individuals that received combined nicotine and mecamylamine treatment when compared with those that received nicotine only (20%), mecamylamine only (15%) or placebo (15%).

While these studies support the use of mecamylamine as an adjunct therapy with NRT for smoking cessation, the studies were limited by small numbers of subjects ($n = 20-48$). In a larger study, subjects ($n = 233$) received nicotine via transdermal patch (21 mg/24 hr) and/or mecamylamine (10 mg/day, oral), administered separately or concurrently, and the reward produced by cigarette smoking was rated using a 7-point scale that ranged from “not at all” to “extremely” (Rose and Behm, 2004). Results showed that nicotine alone, mecamylamine alone and the combined nicotine-mecamylamine treatment reduce smoking reward to the same degree. In agreement, another large ($n = 180$) study reported that the mecamylamine and nicotine combination therapy was no better than NRT alone in improving the chance of successfully quitting smoking (Glover et al., 2007). One explanation for these conflicting results may be that NRT produces nAChR desensitization, which leads to functional antagonism of the receptors, providing results analogous to those found following mecamylamine combination therapy (Gentry and Lukas, 2002; Quick and Lester, 2002; Picciotto et al., 2008). Thus, mecamylamine does not appear to be an effective adjunct therapy to NRT.

4.2 Alcohol Addiction

Similar to findings in the preclinical literature, reports from clinical studies indicate that mecamylamine has potential as a medication for the treatment of alcohol addiction. Mecamylamine decreased the rewarding effect of alcohol, alcohol-induced mood alterations

and the self-reported desire to consume alcoholic beverages (Blomqvist et al., 2002). Self-report measures included the Drug Effect Questionnaire (Holdstock and de Wit, 1998), the Alcohol Sensation Scale (Maisto et al., 1980), and the Biphasic Alcohol Effects Scale (Martin et al., 1993). Mecamylamine also modified the pharmacokinetic profile of alcohol, as evidenced by decreased blood alcohol concentrations (Blomqvist et al., 2002). Furthermore, mecamylamine decreased the stimulant-like effects of alcohol, i.e., mood effects associated with the rising portion of the blood-alcohol concentration curve (Chi and de Wit, 2003; Young et al., 2005). While results from these clinical studies are encouraging, a small number of subjects were employed in each study (20–27 subjects/study), and neither alcohol-dependent subjects nor nicotine-dependent alcohol users were included. As such, the interpretation of these studies is limited. Given that both alcohol and nicotine alter nAChR expression and function (see above), alcohol-dependent individuals that regularly smoke tobacco are likely to have undergone neuroadaptations in response to repeated exposure to both of these abused drugs. Thus, future studies evaluating potential therapeutics, especially nAChR antagonists, for alcohol-dependence should consider pharmacological history as a critical factor in study design and outcome. Nevertheless, these results provide clinical evidence for a role for nAChRs in mediating the reinforcing properties of alcohol, and suggest that nAChR inhibition may provide an effective mechanism for the treatment of alcohol addiction.

4.3 Depression

In the early 1970's, results from clinical studies (Janowsky et al., 1972a, b) led to the "cholinergic hypothesis of depression", which states that increased cholinergic tone is an underlying mechanism of depression. Classical tricyclic antidepressants, as well as newer, more selective monoamine transporter inhibitors, have been shown to act as nAChR antagonists. Inhibition of hypercholinergic activity has been suggested as a potential approach to the treatment for depression (Lippiello et al., 2008; Shytle et al., 2002d). Several clinical trials have evaluated mecamylamine as a treatment for depression. Following 8 weeks of treatment, mecamylamine (2.5 mg/kg/day) decreased reports of "sudden mood changes" in patients with comorbid depression and Tourette's syndrome (Shytle et al., 2002d). Mecamylamine (5 mg/day, orally, for 7 days; 10 mg/day, week 2–8, as tolerated) reduced depressive symptoms, as measured using the 17- and 21-item Hamilton Depression Rating Scale and the Beck Depression Inventory (BDI) II scale (George et al., 2008). While limited by small sample sizes (n = 38 and 21 at the end of treatment, respectively), results from these initial studies investigating the therapeutic benefit of mecamylamine in depression suggest that inhibition of nAChRs may be a novel approach to treatment.

Individuals diagnosed with major depressive disorder that do not respond to multiple antidepressant medications are classified as treatment resistant (Little, 2009). One therapeutic approach for treatment-resistant individuals is augmentation pharmacotherapy, which refers to administration of both non-standard antidepressants in combination with classical antidepressants (e.g., SSRIs), with the aim of enhancing effects of each medication (Carvalho et al., 2009). Mecamylamine has been evaluated as an augmentation therapeutic for depression (Bacher et al., 2009). Results from a recent Phase 2B clinical trial employing 579 subjects showed that the combination of S-(+)mecamylamine (TC-5214) and SSRI citalopram hydrobromide was more effective than citalopram alone, improving the primary outcome measure of depression (HAM-D rating scale) and secondary outcome measures including irritability, disability, cognition, severity of illness and global improvement (Targacept press release, July 15, 2009). Based on these promising results, the efficacy of TC-5214 as an adjunct therapy was evaluated in a series of five Phase III clinical trials employing patients with major depressive disorder who were not adequately responsive to either SSRIs or selective norepinephrine reuptake inhibitors (SNRIs). However, TC-5214,

administered in either a fixed or flexible dosing schedule, in combination with a SSRI or SNRI, failed to show an advantage in comparison with placebo (AstraZeneca Clinical Study Report Synopses, D4130C00004, D4130C00005, D4130C00002, D4130C00003, D4130C00007, 2012).

While this discrepancy in outcome between the Phase II and III clinical trials was reason for disappointment, results of a contemporaneous study (AstraZeneca Clinical Study Report Synopsis, D4131C00001, 2012), which utilized TC-5214 as a monotherapy, provide some insight as to possible reasons for the failure of the S-(+)mecamylamine adjunct therapy to achieve relief of depressive symptoms. Subjects in the studies which employed TC-5214 as adjunct therapy exhibited a high placebo response rate that was not different from responses obtained in the experimental groups. In contrast, placebo response rate was lower in the TC-5214 monotherapy study. Parameter values for patients administered TC-5214 as a monotherapy suggested that a dose-related antidepressant effect may have begun to emerge as reflected by a least squares mean change in the Montgomery-Asberg depression rating scale from a placebo value of -7.6 to -11.2 for 4 mg twice daily dosage of TC-5214. Unfortunately, the TC-5214 monotherapy study may have been halted prematurely based on results from the contemporaneous evaluation of TC-5214 as an adjunct therapy. Since subjects in the placebo group of the adjunct therapy trials were receiving SSRI or SNRI treatment, it may be that manipulation of the serotonergic or noradrenergic circuitry interferes with the antidepressant action of TC-5214. Thus, a future study evaluating the efficacy of TC-5214 as a monotherapy in a group of antidepressant-naïve, depressed individuals may be of value in further determining the therapeutic value of S-(+)mecamylamine. Furthermore, TC-5214 may provide greater relief of depressive symptoms if tobacco smoking history were taken into account, since nicotine exposure decreases the efficacy of mecamylamine.

Extensive analysis of the results from the clinical trials for depression and work from additional preclinical studies led to the hypothesis that TC-5214 may have efficacy in treatment of overactive bladder (Targacept press release, Sep 05, 2012). Overactive bladder is characterized by increased frequency and urgency of urination that can progress to incontinence, and as such can diminish quality of life. Cholinergic receptors of various subtypes are expressed by urinary bladder epithelium and parasympathetic neurons which innervate the bladder, and stimulation of these receptors leads to bladder contraction and the urge to urinate (Beckel et al., 2005; Beckel and Birder, 2012; Biasi et al., 2000). The promising safety and tolerability profile generated by prior clinical trials, in combination with the pharmacokinetic data of TC-5214 which indicate a high degree of renal excretion, suggest that a low dose of S-(+)mecamylamine may be sufficient to treat overactive bladder in patients unable to tolerate currently available medication. Phase IIb clinical trials are expected to begin for this indication in 2013.

4.4 Cognitive and Attentional Spectrum Disorder

Preclinical results demonstrate that nicotine enhances several facets of cognitive function, including learning and memory (Levin, 2002). Due to these beneficial effects of nicotine, clinical research has focused primarily on the development of subtype-specific nAChR agonists as pharmacotherapeutics for diseases that are associated with cognitive impairments. To elucidate the mechanism underlying the procognitive effects of nicotine, mecamylamine has been employed as a nAChR antagonist to demonstrate nAChR involvement in healthy adults and cognitively impaired patients having various diseases (Dumas et al., 2006; Dumas et al., 2010; Green et al., 2005; Sacco et al., 2005; Sacco et al., 2006). Consistent with the idea that nAChR receptor activation improves cognition, an early study employing young, non-smoking males showed that mecamylamine dose-dependently increased errors in a repeated acquisition task, increased the number of false-alarms during

the delay period in a recognition memory task, and increased response latency in both tasks (Newhouse et al., 1992). While a 20 mg dose of mecamylamine elevated the number of errors in a repeated acquisition task in healthy young and elderly subjects, only the elderly subjects showed impairments in learning following the 10 mg dose (Newhouse et al., 1994). Thus, the elderly appear to be more sensitive to the detrimental effects of mecamylamine in this regard. In another study employing young adults, mecamylamine increased latency to respond, but did not alter performance in an attention task, and only impaired delayed recall, but not immediate recall in a memory task (Pickworth et al., 1997). Interestingly, these effects on attention and memory were independent of smoking status. Thus, mecamylamine occupied an apparently sufficient number of receptors to produce detrimental effects, independent of the presence of nicotine.

Clinical trials evaluating mecamylamine for the treatment for attention disorders, including attention-deficit/hyperactivity disorder (ADHD), have been conducted. In the adult population, ADHD has been reported to be associated with tobacco smoking (Pomerleau et al., 1995). Adolescents who self-report symptoms consistent with a diagnosis of ADHD were found to be ~3-times more likely to both initiate tobacco smoking and be current smokers (Tercyak et al., 2002). These observations led to the hypothesis that individuals with ADHD may be self-medicating by smoking tobacco to alleviate their ADHD symptoms (Levin et al., 1996; Potter et al., 2006; Wilens et al., 1999). In support of this hypothesis, nicotine delivered via transdermal patch was found to improve attention similar to methylphenidate, a medication commonly prescribed for ADHD (Levin and Rezvani, 2002). If nicotine is producing beneficial effects in ADHD and the underlying mechanism is nAChR desensitization, mecamylamine may also be effective as a treatment or as an adjunct in attention-related disorders. However, studies evaluating effects of mecamylamine and employing a small number of healthy young subjects ($n = 12-13$) found no improvement in either memory or attention (Ellis et al., 2006; Potter and Newhouse, 2005). In concordance with preclinical work showing procognitive effects of low doses of mecamylamine (Levin et al., 1993; Levin and Caldwell, 2006; Terry et al., 1999), a clinical study evaluated the pharmacotherapeutic effects of mecamylamine as a treatment for attentional impairments in 15 non-smokers with ADHD (Potter et al., 2009). Low doses (< 1 mg/kg) of mecamylamine were administered and behavioral inhibition, recognition memory and delay aversion were assessed. Mecamylamine (0.5 mg) improved recognition memory, with a concurrent reduction in tolerance for delay, indicating that inhibition of nAChRs by low-dose mecamylamine results in procognitive effects. Mecamylamine at low doses may be acting as a mixed agonist/antagonist or nAChR inhibition may be mimicking nicotine-induced receptor desensitization. More recently, a high dose (20 mg) of mecamylamine was found to block the nicotine-induced increase in behavioral inhibition in the stop signal task in highly impulsive subjects diagnosed with ADHD ($n = 11$; Potter et al., 2012). These results suggest that the efficacy of nicotine as a therapeutic for ADHD may be related to nAChR activation, rather than nAChR desensitization.

Autism is an attention-related disorder on the opposite end of the “attentional spectrum” from ADHD. Autistic individuals are considered to be hyper-focused in contrast to those with ADHD, who are considered hypo-focused. Although based primarily on single case studies and anecdotal reports, mecamylamine has been hypothesized to be an efficacious therapy for autism (Dani and Bertrand, 2007; Lippiello, 2006). In a recent investigation, 10 children diagnosed with autism spectrum disorder were administered ascending fixed doses (0.5 mg/day-5 mg/day) of mecamylamine for a total of 18 weeks. Progress according to the Ohio Autism Clinical Impressions Scale was evaluated by a blinded independent clinician (Arnold et al., 2012). No differences were found between subjects receiving placebo versus those receiving mecamylamine. When evaluating the treatment group alone, 40% of the subjects demonstrated improvement that was sustained for the duration of the treatment. Of

those four children, three received a maximum mecamylamine dose of only 0.13–0.15 mg/kg/day. These results illustrate that the beneficial effects of mecamylamine for this application may be experienced only with low doses (< 1 mg/kg).

4.5 Dosage-Related Effects

Review of the literature reveals that the majority of preclinical investigations in which positive effects of mecamylamine (e.g., reduction in drug self-administration, elimination of interoceptive cues associated with drug) were observed, animals were administered moderate to high doses (> 1 mg/kg) of mecamylamine. Furthermore, the majority of clinical studies investigating therapeutic efficacy of mecamylamine also employed moderate to high doses (5–50 mg/day). In contrast, positive therapeutic benefits on cognition were found only following administration of relatively low doses (< 1 mg/kg) of mecamylamine in studies employing animals and humans. Thus, it appears that procognitive effects of mecamylamine manifest at low doses (< 1 mg/kg), whereas moderate to high doses (> 1 mg/kg) are required for therapeutic benefits with respect to addiction-related indications. Difficulties arise relating the doses of mecamylamine (> 1 mg/kg) which decrease addiction related behaviors with concentration of mecamylamine ($IC_{50} = 0.12 \pm 0.04 \mu\text{M}$; results from current review) that inhibit nicotine-evoked dopamine release from brain slices *in vitro*, since concentrations of mecamylamine in brain following peripheral administration *in vivo* have not been reported. However, the dose-related effects of mecamylamine on cognitive and drug abuse-related behaviors may be due to the involvement of distinct brain regions and differing nAChR populations, which exhibit different desensitization recovery rates (Giniatullin et al., 2005). Furthermore, nAChR subtypes display differential sensitivity to mecamylamine (Chavez-Noriega et al., 1997). Although low mecamylamine doses (< 1 mg/kg) are relatively selective for nAChRs (Clarke et al., 1994), a limitation of studies using high mecamylamine doses (> 1 mg/kg) is that such doses are not selective for nAChRs, and NMDA receptor interactions may be contributing to the effects of mecamylamine (Fu et al., 2008; O'Dell and Christensen, 1998; Papke et al., 2001).

5.0 CONCLUSIONS

The preclinical and clinical literature discussed in the current review suggest that mecamylamine may have therapeutic efficacy in a variety of CNS-related disorders. However, since much of the experimental data regarding the beneficial effects of mecamylamine have been obtained either from preclinical studies or clinical trials employing a small number of subjects, a comprehensive evaluation of the potential therapeutic benefit of mecamylamine has not been realized. The coincidence of smoking behavior in patients with depression, ADHD and alcoholism (Covey et al., 1998; Glassman, 1993; Meyerhoff et al., 2006; Pomerleau et al., 1995) leads to the suggestion that these individuals may be self-medicating with nicotine (Levin et al., 1996; Markou et al., 1998; Potter et al., 2006). However, an unresolved question is whether the beneficial effects of nicotine are due to nAChR activation or desensitization. As illustrated by the *in vitro* studies presented herein, nicotine exposure can modify mecamylamine binding to nAChRs. Considering these preclinical findings, future studies evaluating the effects of mecamylamine should consider tobacco smoking status, as well as nicotine replacement therapy (e.g. nicotine patch, nicotine gum, etc.), as an important factor when assessing mecamylamine efficacy. Thus, smoking history and status should be either an exclusionary factor or a covariate when determining the therapeutic benefit of mecamylamine and other nAChR antagonists being evaluated for clinical efficacy.

Although the future of mecamylamine as a therapeutic for the diseases and conditions discussed herein is questionable, the discovery of nAChR antagonists which are nAChR subtype selective holds promise. Both the preclinical and clinical literature indicate that the

therapeutic efficacy of mecamylamine may be limited by anticholinergic side effects (e.g., constipation, hypotension), which result from inhibition of peripheral nAChRs. Furthermore, high doses of mecamylamine may produce cognitive deficits. Since mecamylamine interacts nonselectively with all known nAChR subtypes, the development of nAChR antagonists which exhibit selectivity for a specific nAChR subtype may augment its therapeutic benefits, while eliminating deleterious side-effects associated with mecamylamine. Questions regarding the therapeutic efficacy of subtype-selective nAChR antagonists will be addressed as novel compounds emerge from discovery and are developed to treat diseases in which the pathology is influenced by nicotinic receptors.

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ABBREVIATIONS

ACh	acetylcholine
ADHD	attention deficit hyperactivity disorder
ANOVA	analysis of variance
BSA	bovine serum albumin
CNS	central nervous system
DA	dopamine
dr	dose ratio
FDA	United States Food and Drug Administration
nAChR	nicotinic acetylcholine receptor
SNRI	selective norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor

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Highlights

- Mecamylamine inhibits all nicotinic acetylcholine receptor subtypes
- Mecamylamine used in studies to assess effects on variety of addictions and disorders
- Nicotine alters binding characteristics of mecamylamine
- Researchers should determine smoking history of patients in future trials

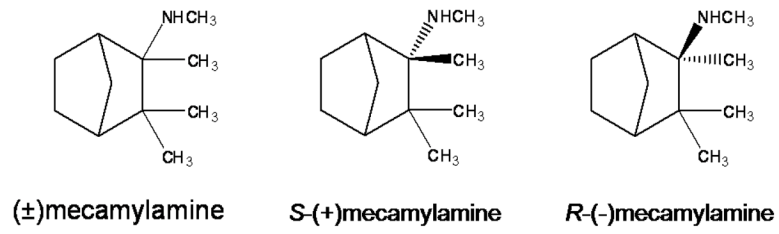


Fig. 1.
Chemical structures of racemic mecamylamine, *S*(+)-mecamylamine and *R*(-)-mecamylamine.

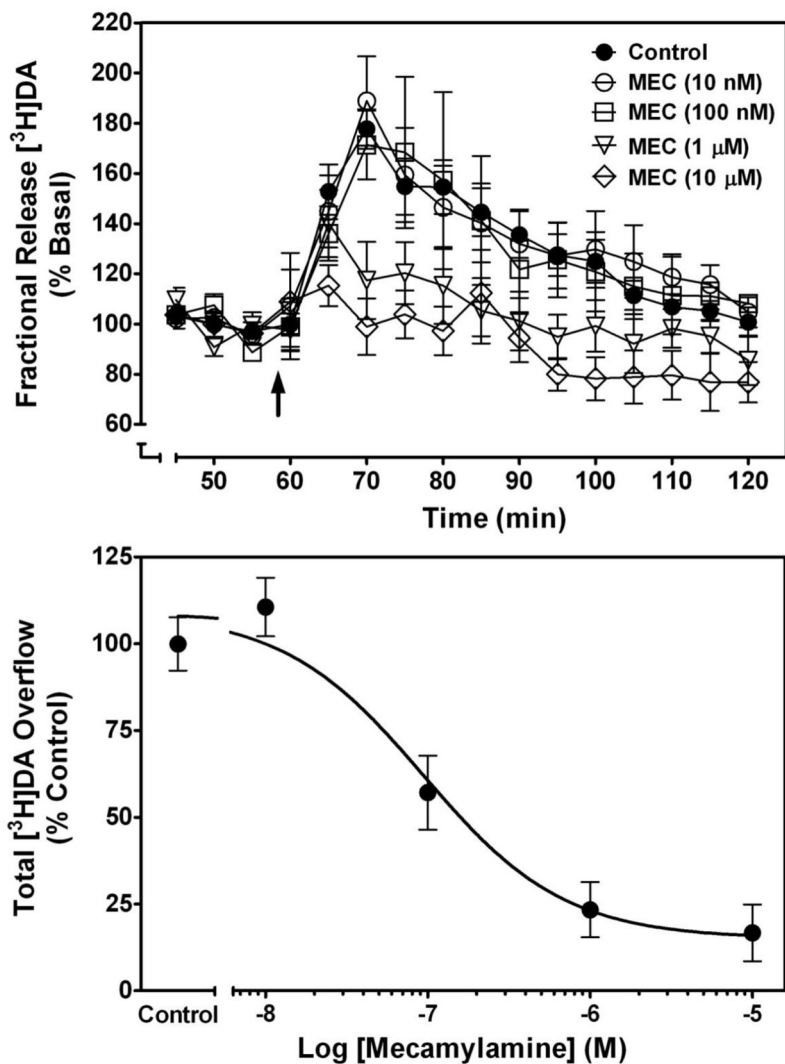


Fig. 2. Mecamylamine inhibits nicotine-evoked [^3H]DA overflow from superfused rat striatal slices in a concentration-dependent manner

Time course (top) of mecamylamine-induced inhibition of nicotine-evoked [^3H]DA overflow. Arrow indicates time point at which nicotine was added to the superfusion buffer. Data are expressed as fractional release as a percentage of basal (mean \pm SEM); $n = 6$ rats. Mean basal [^3H]outflow was 0.75 ± 0.02 fractional release as percentage of tissue [^3H] content. Time course data were used to generate [^3H]DA overflow data (bottom). Control represents [^3H]DA overflow in response to $10 \mu\text{M}$ nicotine (total [^3H]DA overflow as a percentage of tissue [^3H]-content, mean \pm S.E.M.). Control response to nicotine in the absence of antagonist was 3.06 ± 0.24 [^3H]DA overflow. Concentration-response curves were generated using nonlinear regression. Data are expressed as percentage of control; $n = 5/\text{group}$.

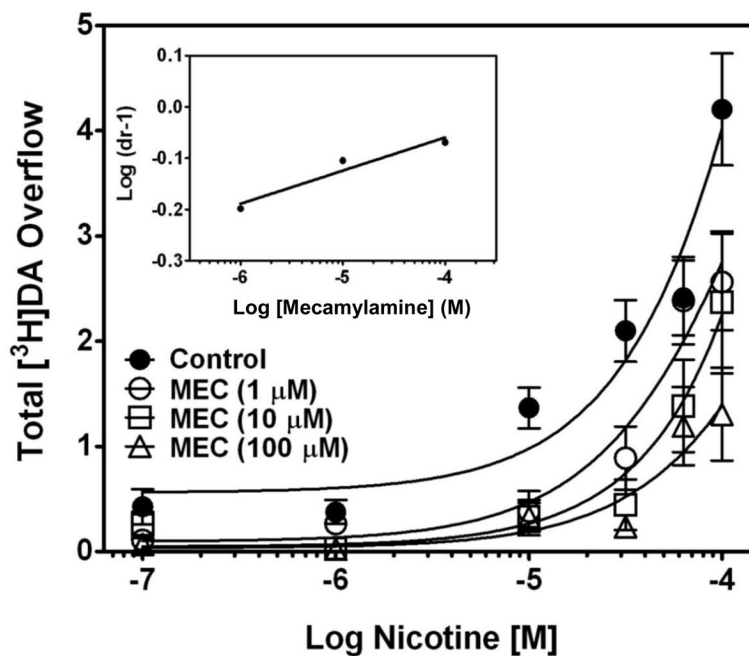


Fig. 3. Schild analysis of mecamylamine inhibition of nicotine-evoked [³H]DA overflow from superfused rat striatal slices

After collection of the third sample, slices were superfused with buffer in the absence or presence of mecamylamine (1, 10, 100 μ M) for 45 min before the addition of nicotine (0.1–100 μ M) to the buffer, and superfusion continued for an additional 45 min. For each nicotine concentration, control response is that for nicotine in the absence of mecamylamine. Control represents [³H]DA overflow in response to nicotine alone (total [³H]DA overflow as a percentage of tissue [³H]-content, mean \pm S.E.M.); $n = 5$ rats/mecamylamine concentration; control, $n = 12$ rats (mecamylamine was between-groups factor, control was contemporaneous with each mecamylamine concentration). Concentration-response curves were generated by nonlinear regression. Inset shows the Schild regression in which the log of $dr-1$ was plotted as a function of log of mecamylamine concentration, and data were fit by linear regression.

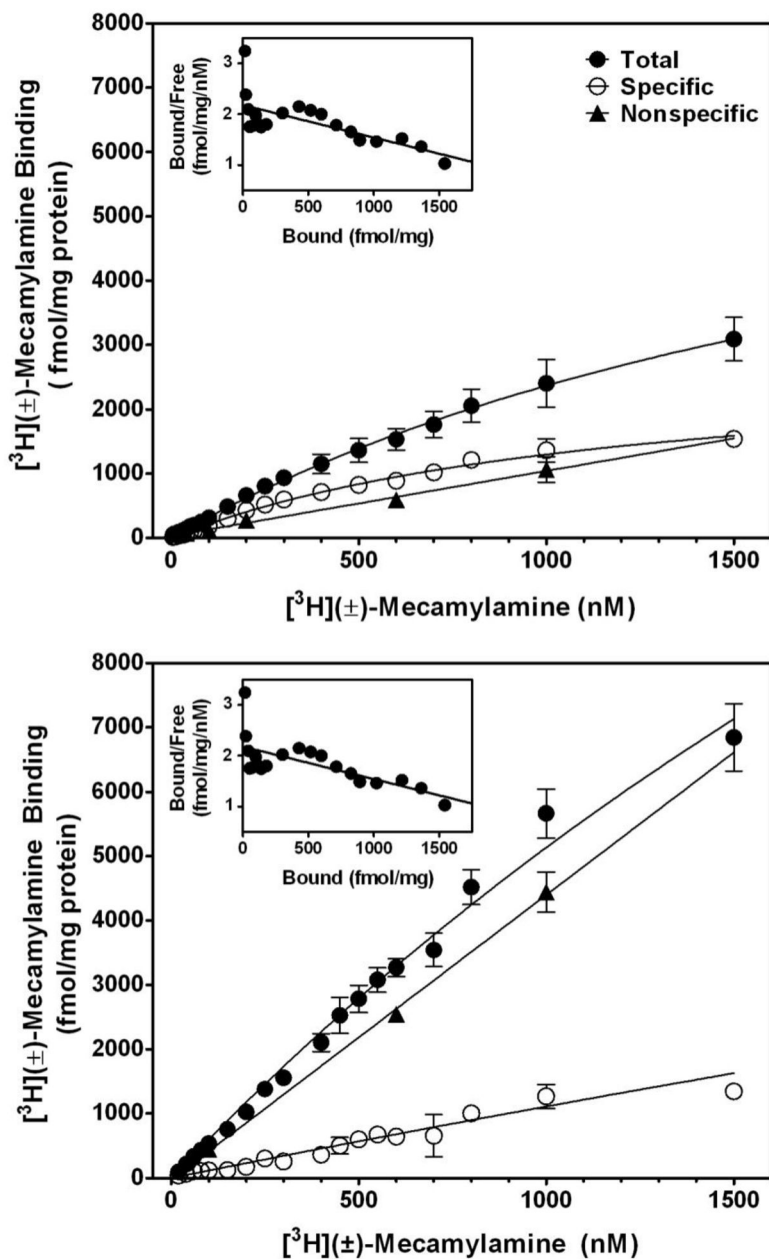


Fig. 4. Saturation analysis of racemic ^3H mecamylamine binding to rat brain membranes in the absence (top) and presence of 1 mM S(-)nicotine (bottom)

Saturation analysis was determined using a concentration range of 20–1500 nM of racemic ^3H -mecamylamine in both the absence (top) and presence of nicotine (1 mM; bottom). Nonspecific binding was measured with 100 μM (\pm)-mecamylamine. Data are expressed as fmol/mg protein and represent the mean \pm S.E.M. of three independent experiments. Curves were generated using nonlinear regression for a one-site model. The inset illustrates the Scatchard transformation of the specific binding data.

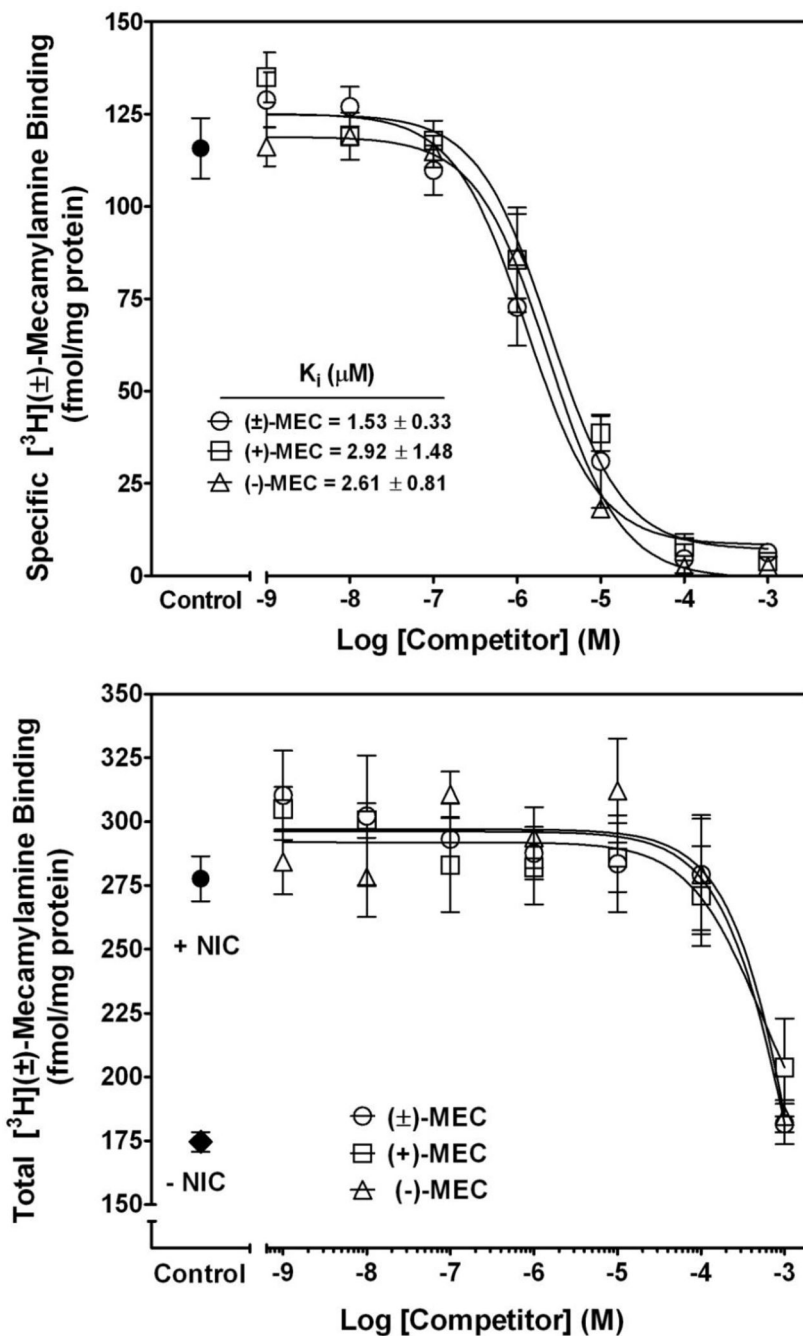


Fig. 5. High concentrations of mecamylamine stereoisomers are required to compete for racemic [³H]-mecamylamine binding to rat brain membranes in the presence of 1 mM S(-)nicotine
Competitive binding assays were performed for racemic, S(+)- and R(-)-mecamylamine (1 nM - 1 mM) against 100 nM racemic [³H]-mecamylamine both in the absence (top) and presence of nicotine (1mM; bottom panel). Nonspecific binding was determined using 100 μM racemic mecamylamine. Data are expressed as fmol/mg protein and represent the mean ± SEM of three independent experiments. Curves were generated by nonlinear regression using a one-site model. K_i values are expressed as mean ± S.E.M. K_i values could not be calculated in the presence of nicotine.