Analysis of Mecamylamine Stereoisomers on Human Nicotinic Receptor Subtypes

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ABSTRACT

Because mecamylamine, a nicotinic receptor antagonist, is used so often in nicotine research and because mecamylamine may have important therapeutic properties clinically, it is important to fully explore and understand its pharmacology. In the present study, the efficacy and potency of mecamylamine and its stereoisomers were evaluated as inhibitors of human $\alpha3\beta4$, $\alpha3\beta2$, $\alpha7$, and $\alpha4\beta2$ nicotinic acetylcholine receptors (nAChRs), as well as mouse adult type muscle nAChRs and rat N-methyl-D-aspartate (NMDA) receptors expressed in Xenopus oocytes. The selectivity of mecamylamine for neuronal nAChR was manifested primarily in terms of slow recovery rates from mecamylamine-induced inhibition. Neuronal receptors showed a prolonged inhibition after exposure to low micromolar concentrations of mecamylamine. Muscle-type receptors showed a transient inhibition by similar concentrations of mecamylamine, and NMDA receptors were only transiently in-

hibited by higher micromolar concentrations. Mecamylamine inhibition of neuronal nAChR was noncompetitive and voltage dependent. Although there was little difference between S-(+)-mecamylamine and R-(−)-mecamylamine in terms of 50% inhibition concentration values for a given receptor subtype, there appeared to be significant differences in the off-rates for the mecamylamine isomers from the receptors. Specifically, S-(+)-mecamylamine appeared to dissociate more slowly from $\alpha4\beta2$ and $\alpha3\beta4$ receptors than did R-(−)-mecamylamine. In addition, it was found that muscle-type receptors appeared to be somewhat more sensitive to R-(−)-mecamylamine than to S-(+)-mecamylamine. Together, these findings suggest that in chronic (i.e., therapeutic) application, S-(+)-mecamylamine might be preferable to R-(−)-mecamylamine in terms of equilibrium inactivation of neuronal receptors with decreased side effects associated with muscle-type receptors.

Mecamylamine is widely used as a broad-spectrum antagonist of neuronal nicotinic acetylcholine (ACh) receptors (nAChRs) and, despite its common usage, questions still exist as to its precise specificity and mechanisms of action. The present study evaluates the effects of mecamylamine on a wide range of nAChRs and additionally evaluates whether the stereoisomers of this drug augment the specificity or efficacy for desired therapeutic effects.

In the context of therapeutics, mecamylamine (Inversine) was developed and characterized by Merck (Darmstadt, Germany) as a ganglionic blocker with clinically significant hypotensive actions (Stone et al., 1956). Unique characteristics of mecamylamine, including exceptional oral efficacy, rapid onset, long duration of action, and nearly complete absorption from the gastrointestinal tract, made mecamylamine, at that time, a more desirable antihypertensive medication than the existing ganglionic blockers (Baer et al., 1956).

Despite mecamylamine's efficacy in the treatment of hy-

pertension, its side effects resulting from broad parasympathetic inhibition at antihypertensive doses reduced its popularity in the treatment of essential hypertension. However, in addition to its peripheral ganglionic blocking actions, mecamylamine crosses the blood-brain barrier and functions as a cholinergic antagonist specific to neuronal nicotinic receptor subtypes (Varanda et al., 1985; Martin et al., 1989, 1993; Banerjee et al., 1990). Because mecamylamine readily blocks most of the physiological, behavioral, and reinforcing effects of nicotine, it has become the most commonly used of all neuronal nAChR-specific inhibitors in basic nicotine research (Martin et al., 1989, 1993) and has been reported to be effective as an aid to smoking cessation (Tennant et al., 1984; Rose et al., 1994, 1998).

Since nicotine has both agonist and antagonistic properties, nicotinic receptor antagonists, such as mecamylamine, may also be useful for a wide range of nicotine-responsive neuropsychiatric disorders. For example, mecamylamine, like nicotine, has also been reported to possibly augment neuroleptic therapy of Tourette's syndrome (Sanberg et al., 1998). In addition, growing evidence suggests that nicotinic

ABBREVIATIONS: ACh, acetylcholine; nAChR, nicotinic acetylcholine receptor; EC_{10}, EC_{15}, EC_{30}, and EC_{50}, 10%, 15%, 30%, and 50% effective concentrations; IC_{50}, 50% inhibitory concentration; NMDA, N-methyl-D-aspartate.

receptor activation may also be involved with the actions of other drugs of abuse, including psychostimulants and alcohol. It has been reported that mecamylamine prevents the induction of locomotor sensitization to amphetamine in mice (Karler et al., 1996), and low doses of mecamylamine were recently found to reduce cue-induced craving in human cocaine abusers (Reid et al., 1999). Furthermore, several preclinical studies have reported that mecamylamine reduces the behavioral and reinforcing effects of alcohol in animals (Blomqvist et al., 1996, 1997; Ericson et al., 1998; Nadal et al., 1998).

The present study investigates the selectivity of mecamylamine and its stereoisomers by using the genes cloned from the nervous system, which code for nicotinic acetylcholine receptor subunits. The nAChRs are known to be formed by the association of five monomeric subunits. The neuronal nAChR gene family include nine proteins designated as α subunits ($\alpha2$–$\alpha10$) and three proteins designated as β subunits ($\beta2$–$\beta4$) (Papke, 1993; Elgoyhen et al., 1994, 2001). Except for $\alpha7$ subunits that may form functional homomeric receptors, most nicotinic receptors found in the mammalian central nervous system require at least one type of α and one type of β subunit to form functional nAChR channels. Neuronal nAChRs are both structurally and phylogenetically related to the nicotine receptors of the neuromuscular junction. The mature muscle-type nAChRs contain two $\alpha1$ subunits, one $\beta1$ subunit, one δ subunit, and one ϵ subunit (Mishina et al., 1986).

This study specifically evaluated the efficacy and potency of highly pure R-($-$)-mecamylamine and S-($+$)-mecamylamine on human $\alpha3\beta4$, $\alpha4\beta2$, $\alpha3\beta2$, and $\alpha7$ receptors expressed in *Xenopus* oocytes and compared their activity to that of racemic mecamylamine. The voltage dependence and reversibility of inhibitory activity was also investigated. Since prior research in this lab suggested that a residual inhibition of receptors by mecamylamine is still present after a 5-min wash period (Webster et al., 1999), it was hypothesized that the persistence of block (an indirect indication of the dissociation rate) might prove to be an important distinguishing feature of the stereoisomers. In addition, the report of finding "interesting differences" between the actions of mecamylamine stereoisomers in assays measuring neuromuscular transmission (Schoenenberger et al., 1986) prompted our investigation of the inhibitory actions of these stereoisomers at adult-type mouse muscle nAChRs expressed in *Xenopus* oocytes.

Materials and Methods

Chemicals. Fresh acetylcholine (Sigma, St. Louis, MO) stock solutions were made daily in Ringer's solution and diluted. Racemic-($\pm$)-mecamylamine (N-2,3,3-tetramethylbicyclo[2.2.1]heptan-2-amine) and the stereoisomers were supplied by Layton Biosciences (Menlo Park, CA). All other chemicals for electrophysiology were obtained from Sigma (St. Louis, MO).

Preparation of RNA and Expression in Xenopus Oocytes. Mature (>9 cm) female *Xenopus laevis* African toads (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Before surgery, frogs were anesthetized by placing the animal in a 1.5 g/l solution of MS222 (3-aminobenzoic acid ethyl ester) for 30 min. Eggs were removed from an incision made in the abdomen. The incisions were disinfected with gentamicin, sutured with 4-0 gut, and the animals were allowed to recover from the anesthesia in a humid environment. Postoperative animals were kept in isolation tanks and checked daily before return to the colony.

After linearization and purification of cloned cDNA, RNA transcripts were prepared in vitro using the appropriate mMessage mMachine kit from Ambion, Inc. (Austin, TX). Harvested oocytes were treated with collagenase from Worthington Biochemical Corporation (Freehold, NJ) for 2 h at room temperature in calcium-free Barth's solution (88 mM NaCl, 10 mM HEPES, pH 7.6, 0.33 mM $MgSO_4$, 0.1 mg/ml gentamicin sulfate). Subsequently, stage 5 oocytes were isolated and injected with 50 nl each of a mixture of the appropriate subunit cRNAs. Recordings were made 1 to 7 days after injection, depending on the cRNAs being tested.

Electrophysiology. Oocyte recordings were made with a Warner Instruments (Hamden, CT) OC-725C oocyte amplifier and RC-8 recording chamber interfaced to a Macintosh personal computer. Data were acquired using Labview software (National Instruments, Austin, TX) and filtered at a rate of 6 Hz. Oocytes were placed in the recording chamber with a total volume of about 0.6 ml and perfused at room temperature by frog Ringer's solution (115 mM NaCl, 2.5 mM KCl, 10 mM HEPES, pH 7.3, 1.8 mM $CaCl_2$) containing 1 μM atropine to inhibit potential muscarinic responses. A Mariotte flask filled with Ringer's solution was used to maintain a constant hydrostatic pressure for drug deliveries and washes. Current electrodes were filled with a solution containing 250 mM CsCl, 250 mM CsF, and 100 mM EGTA and had resistances of 0.5 to 2 MΩ. Voltage electrodes were filled with 3 M KCl and had resistances of 1 to 3 MΩ.

Drugs were diluted in perfusion solution and loaded into a 2-ml loop at the terminus of the perfusion line. A bypass of the drug-loading loop allowed bath solution to flow continuously while the drug loop was loaded, and then drug application was synchronized with data acquisition by using a two-way electronic valve. The rate of bath solution exchange and all drug applications was 6 ml/min. We have previously shown that this protocol delivers a brief but essentially complete solution exchange around the surface of the oocyte (Papke and Thinschmidt, 1998). A double-loop protocol was used for the determination of concentration-response relationships. With this method, the cells were first equilibrated for 12 s in a solution of the inhibitor alone and then tested with the coapplication of ACh and the inhibitor.

Experimental Protocols and Data Analysis. Current responses to drug application were studied under two-electrode voltage clamp at a holding potential of -50 mV unless otherwise noted. Holding currents immediately before agonist application were subtracted from measurements of the peak response to agonist. All drug applications were separated by a wash period of 5 min unless otherwise noted. At the start of recording, all oocytes received two initial control applications of ACh. Subsequent drug applications were normalized to the second ACh application to control for the level of channel expression in each oocyte. The second application of control ACh was used to minimize the affects of rundown that occasionally occurred after the initial ACh-evoked response. To measure residual inhibitory effects, an experimental application of ACh with inhibitor or of inhibitor alone was followed by a second application of ACh alone and compared with the preapplication control ACh response. Means and standard errors (S.E.M.) were calculated from the normalized responses of at least four oocytes for each experimental concentration.

For each of the receptor subtypes tested, a control ACh concentration was selected that was sufficient to stimulate the receptors to a level representing a reasonably high value of p_{open} at the peak of the response while minimizing rundown with successive ACh applications. For potent use-dependent inhibitors, we have found that such conditions were adequate to achieve maximal inhibition (Papke et al., 1994; Francis and Papke, 1996). The control ACh concentrations used were 30 μM ACh for $\alpha4\beta2$, 100 μM ACh for $\alpha3\beta4$, 30 μM ACh for $\alpha3\beta2$, 300 μM ACh for $\alpha7$, and 3 μM ACh for $\alpha1\beta1\delta\epsilon$. These correspond to the EC_{30}, EC_{10}, EC_{15}, EC_{50}, and EC_{50}, respectively, for these receptors.

For concentration-response relations, data were plotted using Ka-

leidagraph 3.0.2 (Abelbeck Software; Reading, PA), and curves were generated from the Hill equation (Webster et al., 1999):

$$\text{Response} = \frac{I_{\max}[\text{agonist}]^n}{[\text{agonist}]^n + (\text{EC}_{50})^n}$$

where $I_{\max}$ denotes the maximal response for a particular agonist/subunit combination, and n represents the Hill coefficient. Negative Hill slopes were applied for the calculation of IC_{50} values.

For the analysis of ACh concentration-response relationships in the presence and absence of mecamylamine (i.e., competition experiments), data were initially normalized based on control ACh responses, as described above. Data were subsequently scaled by a factor relating the efficacy of control ACh responses to the maximum responses obtained with ACh.

Calculations of net charge during evoked responses were made by integration of the current responses for 200 s after the initial deflection from baseline. Specifically, raw data values for experimental responses were imported into a template Excel spreadsheet along with the raw data for the corresponding ACh controls obtained 5 min before the experimental response. Each record included a short (0.5 s) interval of baseline that was used for offset correction. Peaks and areas were then calculated for both the experimental and control responses, and the experimental values were expressed relative to their respective controls.

For experiments assessing voltage-dependence of inhibition, oocytes were voltage-clamped at a holding potential of either -40 mV or -90 mV, and a control application of ACh alone was delivered. The holding potential was then kept at the designated voltage for the coapplication of ACh with mecamylamine. Residual inhibition was evaluated with a subsequent application of ACh alone at the test potential, after a 5-min wash period.

Results

Inhibition of nAChR Subtypes by Mecamylamine Stereoisomers. Racemic mecamylamine and its stereoisomers were tested for their ability to inhibit the ACh-evoked responses of $\alpha4\beta2$, $\alpha3\beta4$, $\alpha3\beta2$, and $\alpha7$ type receptors expressed in *Xenopus* oocytes. Raw data traces showing the effect of 10 μM mecamylamine stereoisomers on these receptor subtypes are shown in Fig. 1. To prevent confounding the effect of changing both ACh and mecamylamine concentrations during the rising phase of the evoked responses, cells were first pre-equilibrated with mecamylamine before ACh was applied in the continuing presence of mecamylamine (see *Materials and Methods*). As shown in Fig. 1, 10 μM of either mecamylamine stereoisomer produced significant inhibition of the nAChR subtypes. Additionally, for each of the β subunit-containing receptor subtypes, there was a marked decrease in subsequent control responses to ACh when it was applied 5 min after exposure to mecamylamine. A summary of the residual inhibition produced by 10 μM mecamylamine is shown in Fig. 2. The residual inhibition was greatest for $\beta2$-containing receptors and least for $\alpha7$ receptors. There was no stereo-selectivity apparent in the 5-min recovery data. The time dependence of recovery is considered further below.

The concentration-response analyses for human $\alpha4\beta2$, $\alpha3\beta4$, $\alpha3\beta2$, and $\alpha7$ nAChR are shown in Fig. 3. The IC_{50} values are given in Table 1. The mecamylamine compounds were most potent at inhibiting $\alpha3\beta4$ receptors and least potent at inhibiting $\alpha7$ receptors. There appeared to be very little difference between the R-$(-)$-isomers and S-$(+)$-isomers at any of these receptor subtypes in terms of potency for inhibition.

The Selectivity of Mecamylamine for Neuronal nAChR. To confirm the selectivity of mecamylamine for neuronal nAChR over muscle-type nAChR, we also examined the effects of R-$(-)$-mecamylamine and S-$(+)$-mecamylamine on adult-type $\alpha1\beta1\delta\epsilon$ receptors, using mouse cDNA clones. Mecamylamine could produce a transient inhibition of muscle-type receptor responses with a potency roughly comparable with that for the inhibition of $\alpha4\beta2$ receptors (Fig. 4 and Table 1). There was a tendency for R-$(-)$-mecamylamine to produce more inhibition of muscle-type receptors than S-$(+)$-mecamylamine; however the difference was only significant at the 1 μM concentration (unpaired t test $p < 0.05$). In contrast to the inhibition of β subunit-containing neuronal receptors, the inhibition of muscle receptors was fully reversed after a 5-min wash (Fig. 4B).

The effects of R-$(-)$-mecamylamine and S-$(+)$-mecamylamine were also evaluated on oocytes coexpressing the NMDA receptor subunits NR1 and NR2b. The NR1 subunit, which is ubiquitous throughout the brain, produces robust functional responses when coexpressed with the NR2b subunit and activated by glutamate and the coagonist glycine (Fig. 5). The NR2b subunit in vivo is selectively present in the forebrain with high levels of expression in the cerebral cortex and hippocampus, as well as the septum, caudate putamen, and olfactory bulb (Ozawa et al., 1998), making the combination of NR2b with NR1 relevant for both cognitive and motor functions in the central nervous system. As shown in Fig. 5, whereas both mecamylamine stereoisomers applied at a concentration of 100 μM could produce a transient inhibition of NMDA receptor responses to the coapplication of 10 μM glutamate + 10 μM glycine ($p < 0.001$), this effect was reversible after a 5-min wash. NMDA receptor responses obtained in the presence of mecamylamine were also analyzed in terms of net charge. Analysis of net charge for neuronal nAChRs tended to show a somewhat greater effect than analysis of peak current (see below), whereas for NMDA receptors inhibition of net charge was no greater than inhibition of peak currents (Fig. 5A). The rapid reversibility of mecamylamine block was apparent in the response rebounds seen as mecamylamine was washed from the bath (Fig. 5B).

Recovery Time Course. Since significant residual inhibition was detected after a 5-min washout for all neuronal nAChR other than $\alpha7$, we conducted recovery time course experiments in which we obtained an initial inhibition of greater than 50% with the coapplication of 10 μM R-$(-)$-mecamylamine or S-$(+)$-mecamylamine and ACh at the control concentration (see above), and then followed the recovery of response with control ACh applications repeated at 5-min intervals. As shown in Fig. 6, for $\beta2$-containing receptors, recovery from mecamylamine-induced inhibition seemed to follow simple exponential kinetics. For $\alpha3\beta2$ receptors, both R-$(-)$-mecamylamine and S-$(+)$-mecamylamine had time constants of recovery of about 33 $\pm$ 4 min, which was similar to the time constant of recovery for S-$(+)$-mecamylamine at $\alpha4\beta2$. The τ for R-$(-)$-mecamylamine on $\alpha4\beta2$ receptors was somewhat faster (23 $\pm$ 1.2 min). Note that although inhibition of $\alpha4\beta2$ receptors during the initial coapplication was not significantly different between cells treated with R-$(-)$-mecamylamine or S-$(+)$-mecamylamine, at each time point represented in Fig. 6A there was significantly less residual inhibition for the cells treated with R-$(-)$-mecamylamine
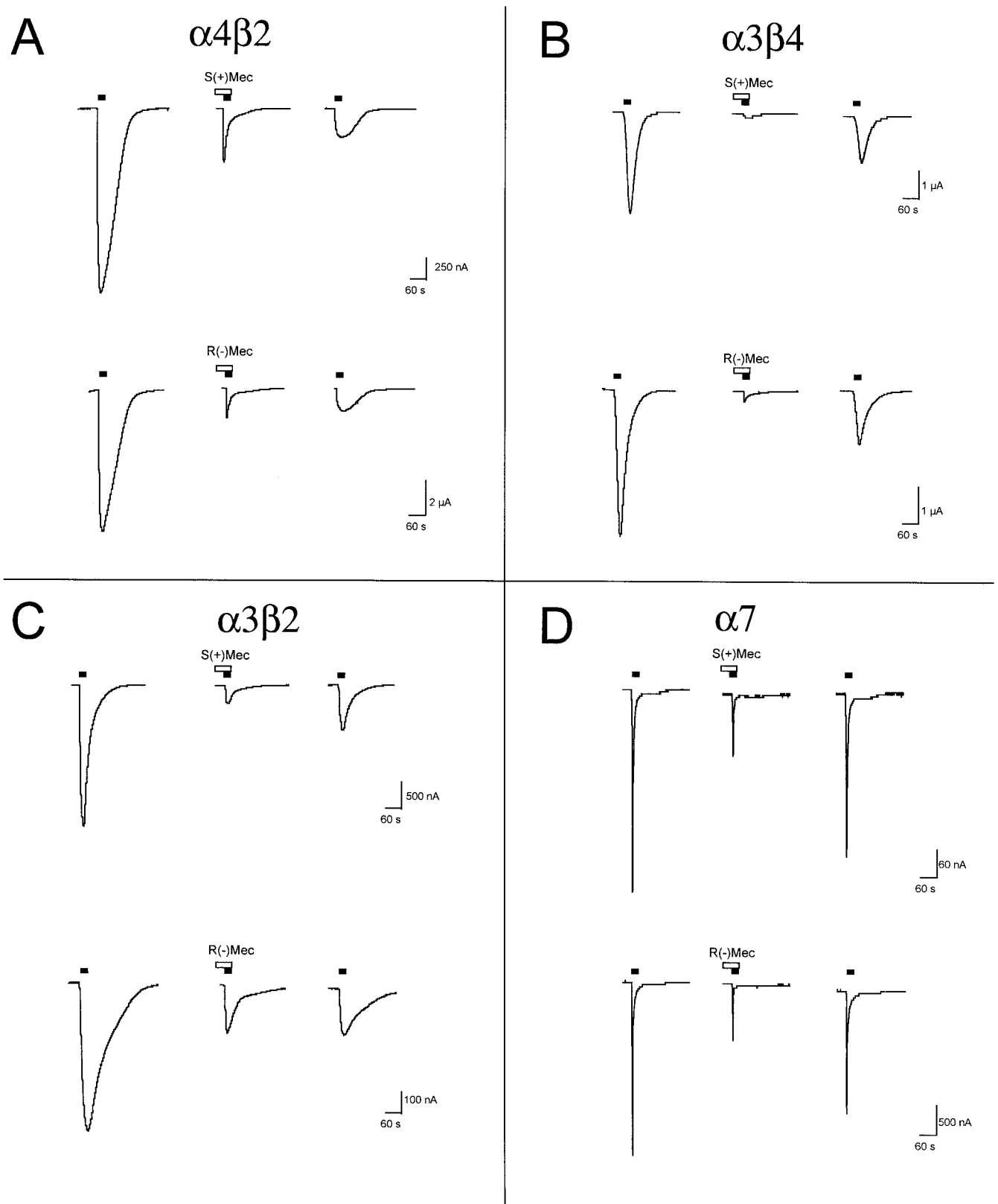
Fig. 1. Effect of mecamylamine (Mec) on neuronal nAChR. A, representative traces showing the effect of the two mecamylamine stereoisomers on ACh-evoked currents in $\alpha4\beta2$-expressing oocytes. The traces illustrate initial control responses to 30 μM ACh alone (application indicated by black bar) and then the application of 30 μM ACh in the presence of 10 μM mecamylamine (open bar). B, representative traces showing the effect of the two mecamylamine stereoisomers (10 μM) on currents evoked by 100 μM ACh in $\alpha3\beta4$-expressing oocytes. C, representative traces showing the effect of the two mecamylamine stereoisomers (10 μM) on currents evoked by 30 μM ACh in $\alpha3\beta2$-expressi
ng oocytes. D, representative traces showing the effect of the two mecamylamine stereoisomers (10 μM) on currents evoked by 300 μM ACh in $\alpha7$-expressing oocytes. In each panel, the third trace in each row shows the response to a control ACh application 5 min after the application of mecamylamine.
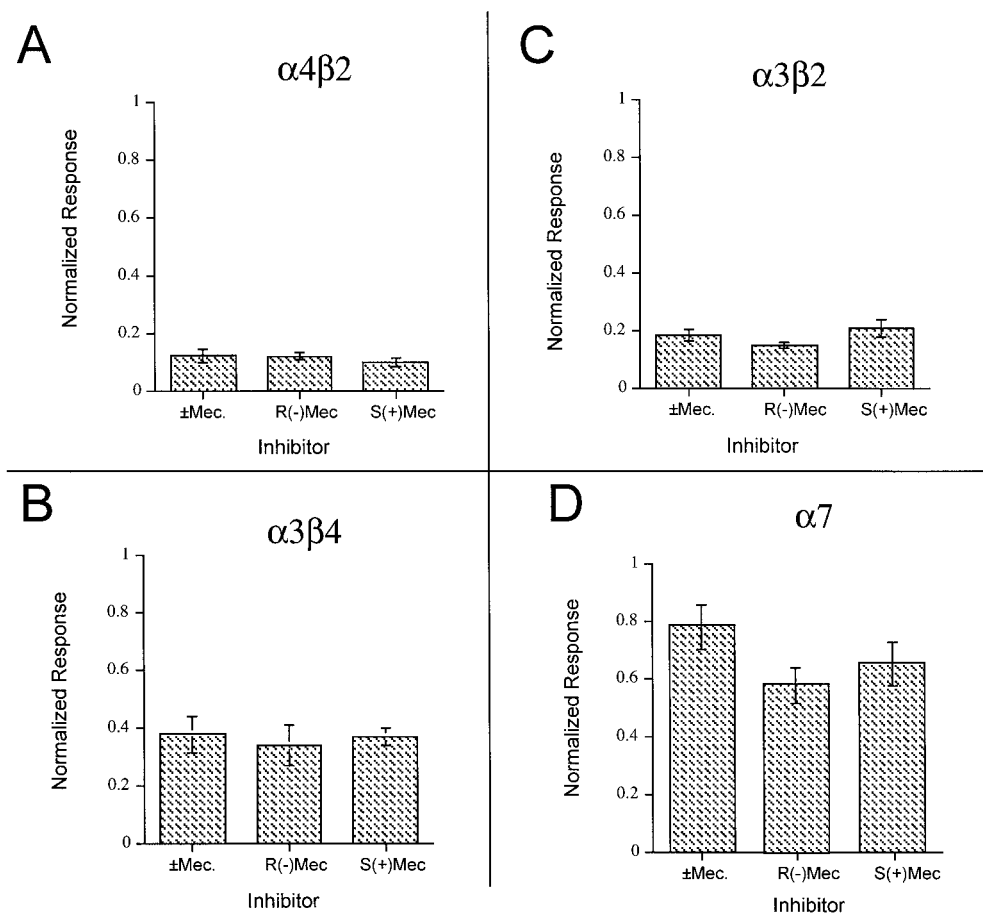
Fig. 2. Residual effects of mecamylamine (Mec) on ACh control responses. Control ACh responses 5 min after the coapplication of 10 μM mecamylamine to $\alpha4\beta2$ receptors (A), $\alpha3\beta4$ receptors (B), $\alpha3\beta2$ receptors (C), and $\alpha7$ receptors (D). Control ACh concentrations used for these receptor subtypes were 30 μM, 100 μM, 30 μM, and 300 μM, respectively. Each bar represents the average normalized responses of four to seven cells.

than with S-(+)-mecamylamine ($p < 0.05$ at 5 and 25 min, and $p < 0.01$ at other time points).

In contrast to the $\beta2$-containing receptors, the recovery of $\alpha3\beta4$ receptors did not follow simple exponential kinetics. There appeared to be a fast phase over the first 15 min ($\tau = 19 \pm 3.4$ min and 12 ± 1.3 min for S-(+)-mecamylamine and R-(−)-mecamylamine, respectively). However, after the first 15 min, no further recovery was observed. This would suggest that mecamylamine may exert two qualitatively different forms of inhibition on $\alpha3\beta4$ receptors.

Competition Studies. To determine whether mecamylamine produced inhibition of neuronal nAChR through a mechanism that is noncompetitive with ACh, we conducted ACh concentration-response studies of $\alpha4\beta2$ and $\alpha3\beta4$ receptors in the absence or presence of either S-(+)-mecamylamine or R-(−)-mecamylamine (Table 2). Data were analyzed both in terms of peak currents and in terms of the net charge during the entire evoked response (see *Materials and Methods*). Data were normalized to the peak or net charge of corresponding control ACh responses. As shown in Fig. 7, both forms of analysis indicate that the inhibition produced by the mecamylamine stereoisomers was not surmounted by increasing the ACh concentration and that the relative amount of inhibition produced by a fixed concentration of mecamylamine was relatively constant over a wide range of ACh concentrations.

We also evaluated whether mecamylamine behaved as a partial agonist for the neuronal nAChR subtypes tested. S-(+)-mecamylamine and R-(−)-mecamylamine were applied to receptors over a wide range of concentrations (10 nM–100 μM) in the absence of ACh. No agonist activity was detected within the limits of our systems' sensitivity (approximately 0.1% of the ACh controls, data not shown).

Voltage Dependence of Inhibition. We evaluated the voltage dependence of inhibition by R-(−)-mecamylamine and S-(+)-mecamylamine in coapplication experiments. Specifically, cells were held at either −40 mV or −90 mV and tested for their responses to control concentrations of ACh (see above). After a 5-min wash, mecamylamine was coapplied with ACh. Cells were then washed for 5 min and tested again for their response to a second control ACh application. Cells were held at the indicated holding potential throughout the entire procedure. Through the use of a coapplication protocol rather than a preincubation procedure (as with the concentration-response curve experiments described above), we can evaluate both the voltage dependence of the onset of inhibition (Fig. 8, $t = 0$ data) and the voltage dependence of recovery, presumably representing the off-rates of the drugs (Fig. 8, $t = 5$ data). Note that the data in Fig. 8 represent the effects of mecamylamine on amplitude of the peak currents relative to the amplitude of the peak currents of control applications of ACh before mecamylamine was applied. The mecamylamine concentrations tested in these coapplication experiments were 10 μM for $\alpha7$ receptors, 5 μM for $\alpha4\beta2$ and $\alpha3\beta2$ receptors, and 1 μM for $\alpha3\beta4$ receptors. As
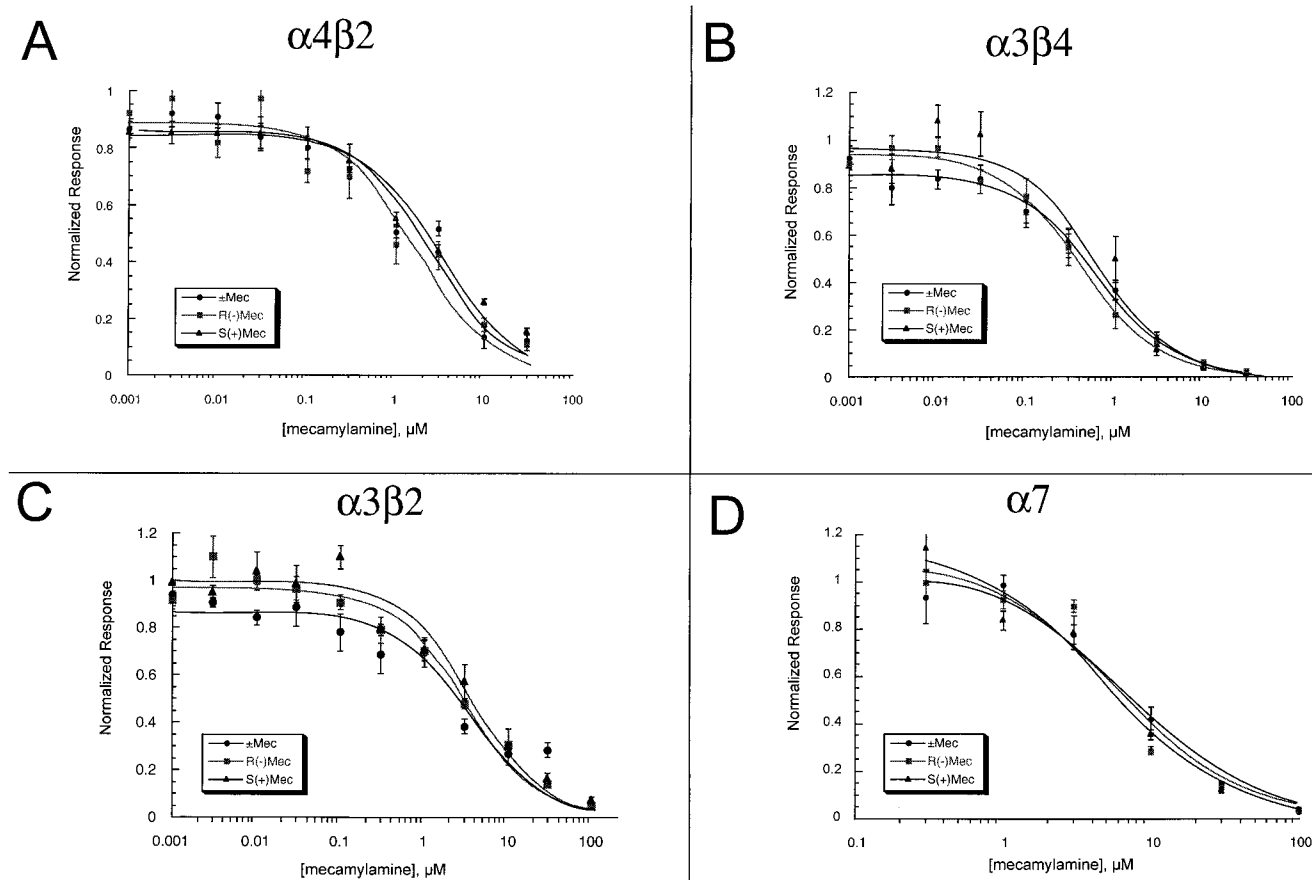
Fig. 3. Concentration-response relationships for the effects of mecamylamine (Mec) and its stereoisomers on neuronal nAChR. A, concentration-response relationship for mecamylamine's inhibition of the peak currents of $\alpha4\beta2$ receptors when 30 μM ACh was coapplied with mecamylamine at the indicated concentrations. Data from each oocyte were normalized to that cell's response to 30 μM ACh alone. B, concentration-response relationship for mecamylamine's inhibition of the peak currents of $\alpha3\beta4$ receptors when 100 μM ACh was coapplied with mecamylamine at the indicated concentrations. Data from each oocyte were normalized to that cell's response to 100 μM ACh alone. C, concentration-response relationship for mecamylamine's inhibition of the peak currents of $\alpha3\beta2$ receptors when 30 μM ACh was coapplied with mecamylamine at the indicated concentrations. Data from each oocyte were normalized to that cell's response to 30 μM ACh alone. D, concentration-response relationship for mecamylamine's inhibition of the peak currents of $\alpha7$ receptors when 300 μM ACh was coapplied with mecamylamine at the indicated concentrations. All cells were pre-equilibrated with mecamylamine, at the indicated concentration, for 10 s before the coapplication of agonist with antagonist. Each point represents the average normalized response of four to seven cells.

TABLE 1

IC$_{50}$ values

Receptor	Racemic Mecamylamine	R-$(-)$-Mecamylamine	S-$(+)$-Mecamylamine
$\alpha3\beta4$	640–90 nM	420–50 nM	640–200 nM
$\alpha4\beta2$	2.5–0.6 μM	1.7–0.5 μM	3.2–0.5 μM
$\alpha3\beta2$	3.6–1.2 μM	3.2–0.6 μM	3.7–0.8 μM
$\alpha7$	6.9–1.6 μM	5.8–2.2 μM	4.6–1.2 μM
$\alpha1\beta1\delta\epsilon$	N.A.	1.1–0.3 μM	2.2–0.6 μM

N.A., not available.

shown in Fig. 8 (A and B), there was significant voltage dependence for the off-rate of both R-$(-)$ and S-$(+)$-mecamylamine with $\alpha4\beta2$ and $\alpha3\beta4$ receptors ($p < 0.01$). Significant effects of voltage ($p < 0.01$) were detected in the $t = 0$ peak responses only for S-$(+)$-mecamylamine on $\alpha7$ (Fig. 8C) and R-$(-)$-mecamylamine on $\alpha3\beta4$ (Fig. 8D). With $\alpha3\beta2$ receptors, only the off-rate of S-$(+)$-mecamylamine showed significant voltage dependence ($p < 0.01$), although the off-rate of R-$(-)$-mecamylamine showed a trend toward significance ($p = 0.0505$). In general, these results suggest that the binding site for mecamylamine may be deep enough into the membrane's electric field to slow the dissociation of mecamylamine when the cell is hyperpolarized.

The results presented in Fig. 8 suggested relatively little effect of voltage on the inhibition of peak currents by mecamylamine. However, with use-dependent inhibitors, the effect of the drug can accumulate throughout the period of activation. Therefore, to further investigate the effect of voltage on the onset of inhibition of neuronal nAChR by mecamylamine, we also analyzed the net charge associated with the coapplication of ACh and mecamylamine at different voltages. As shown in Fig. 9, when net charge was used as the measure of receptor response, significant effects of voltage were detected for the initial inhibition of $\alpha4\beta2$ receptors by both R-$(-)$-mecamylamine and S-$(+)$-mecamylamine, for $\alpha3\beta2$ receptors by S-$(+)$-mecamylamine, and for $\alpha3\beta4$ receptors by R-$(-)$-mecamylamine.

Discussion

Most previous in vitro studies investigating mecamylamine's mode of action have found its inhibitory activity to be voltage-dependent, which suggests that it functions as an open channel blocker (Ascher et al., 1979; Varanda et al., 1985; Fieber and Adams, 1991). Thus, mecamylamine is generally thought of as a noncompetitive antagonist producing
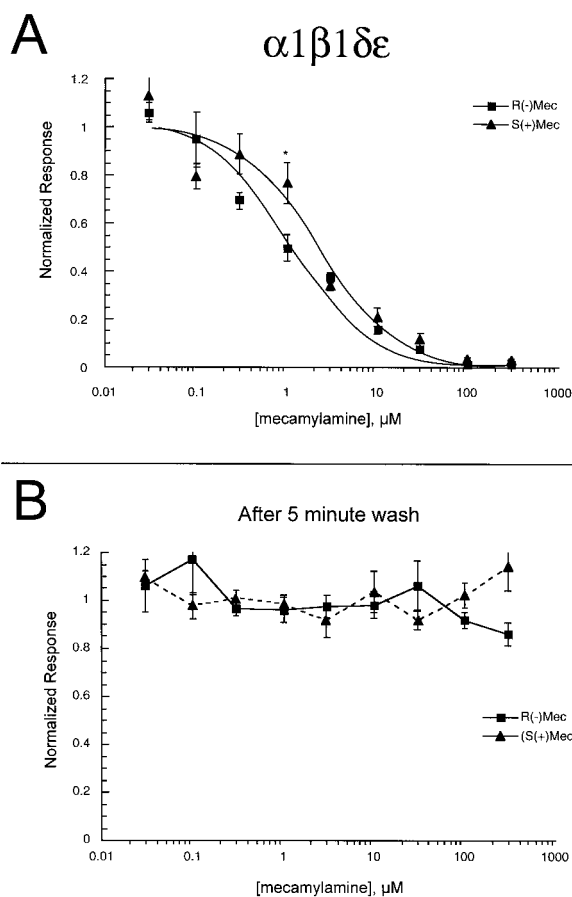
A

$\alpha1\beta1\delta\epsilon$

B

After 5 minute wash

Fig. 4. Effect of mecamylamine (Mec) on ACh-evoked currents in oocytes expressing the $\alpha1\beta1\delta\epsilon$ subunits of the adult muscle-type nAChR. A, concentration-response relationship for mecamylamine's inhibition of the peak currents of muscle-type receptors when 30 μM ACh was coapplied with mecamylamine at the indicated concentrations. Data from each oocyte were normalized to that cell's response to 3 μM ACh alone. All cells were pre-equilibrated with mecamylamine at the indicated concentration for 10 s before the coapplication of agonist with antagonist. Each point represents the average normalized response of at least four cells. B, amplitude of 3 μM ACh control responses obtained 5 min after the application of ACh and mecamylamine at the indicated concentrations. In contrast to the residual inhibition seen with neuronal nAChR receptors, the inhibition of muscle-type receptors was essentially fully reversible.

inhibition by binding to sites other than the agonist activation site (Francis and Papke, 1996). However, Ascher et al. (1979) also found that inhibition of parasympathetic ganglia of the rat was lessened when the drug was coapplied at high agonist concentrations, an observation that is more consistent with a competitive mechanism of inhibition. Moreover, Bertrand et al. (1990) reported that the inhibitory effects of mecamylamine on heterologously expressed chick $\alpha4\beta2$ receptors was voltage independent, yet present only after coapplication with an agonist. Previous research by this lab suggests that mecamylamine's inhibition of rat $\alpha3\beta4$ receptors is voltage dependent, which is consistent with open channel block (Webster et al., 1999). Moreover, analysis of point mutations indicated that residues at the 6' position within the β subunit TM2 domain may be important for mecamylamine's inhibitory properties (Webster et al., 1999).

In a recent study investigating the effects of mecamylamine on human AChRs expressed in *Xenopus* oocytes, Chavez-Noriega et al. (1997) found that a single concentration of mecamylamine (3 μM) produced approximately

50% inhibition of $\alpha2\beta2$, $\alpha4\beta2$, and $\alpha7$ receptors and between 75 and 90% inhibition at $\alpha3\beta4$, $\alpha3\beta2$, $\alpha4\beta4$, and $\alpha2\beta4$ receptors. However, these findings were difficult to interpret since it was not clear which of four possible nicotinic agonists were used for each subunit combination tested, and only a single concentration of mecamylamine was investigated.

Our results indicate that for β subunit-containing receptors, the effects of mecamylamine are long-lived and use-dependent. Although the slow reversibility of mecamylamine's effects is most apparent in our recovery studies, our concentration/response analyses primarily measure the onset of inhibition over a relatively brief period of activation. This implies that our IC_{50} estimates, which are based only on a single period of activation during which inhibition accumulates, are not likely to reflect equilibrium IC_{50} values that, if feasible to measure, would be considerably lower. It may be the case that previous reports, which have suggested higher potency for inhibition by mecamylamine, may have been affected by the accumulation of inhibition with the repeated activation of the receptors. A similar consideration could account for why our apparent IC_{50} values for β subunit-containing neuronal receptors do not differ as much as might be expected from the apparent values for muscle-type receptors. Specifically, since the off-rate of mecamylamine from muscle-type receptors is probably at least 10 times faster than for the neuronal β subunit-containing receptors, our IC_{50} measurements should approximate the equilibrium IC_{50} values for muscle receptors, while underestimating the equilibrium IC_{50} of the β subunit-containing neuronal receptors. Likewise, for the same reason, the rapid reversibility of mecamylamine's block of $\alpha7$ receptors would be consistent with the relatively small effects of mecamylamine on neuronal $\alpha7$-type receptors (Frazier et al., 1998).

In general, previous studies of mecamylamine have used a racemic mixture comprising the optical isomers R-($-$)-mecamylamine and S-($+$)-mecamylamine hydrochloride. The few previous studies aimed at investigating the pharmacology of these two isomers have generally found little or no difference in potency or efficacy. For example, Stone et al. (1962) compared the effects of S-($+$)-mecamylamine hydrochloride with racemic mecamylamine hydrochloride on nicotine-induced convulsions and pupil dilation in mice and found essentially no significant differences between the two compounds. They concluded that "optical isomerism does not play a significant role in determining the degree of activity". Suchocki et al. (1991) also investigated the actions of R-($-$)-mecamylamine and S-($+$)-mecamylamine hydrochloride in assays measuring nicotine-induced depression of spontaneous locomotor activity and antinociception. They found that both optical isomers had similar potency in blocking the antinociception caused by nicotine, whereas the potency of the S-($+$)-mecamylamine isomer in blocking the nicotine-induced depression of spontaneous locomotor activity could not be determined because S-($+$)-mecamylamine, like nicotine, also induced depression of spontaneous locomotor activity. Despite some evidence for agonist activity of the S-isomer of mecamylamine, these investigators also concluded that optical isomerism does not play a significant role in determining the inhibitory activity of mecamylamine. However, Schoenenberger et al. (1986) reported finding "interesting differences" between the actions of R-($-$)-mecamylamine and S-($+$)-mecamylamine hydrochloride in assays measuring
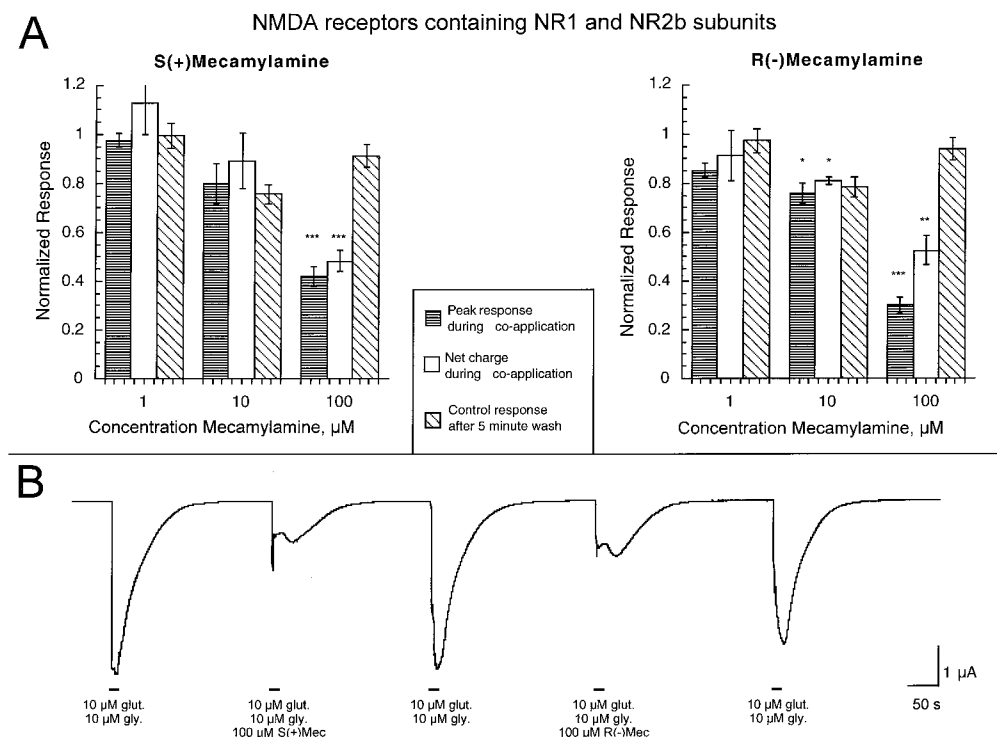
Fig. 5. NMDA receptors containing NR1 and NR2b subunits. Effect of mecamylamine stereoisomers on NMDA receptor currents. Oocytes expressing NR1 and NR2b subunits were tested for the effects of S-(+)-mecamylamine and R-(−)-mecamylamine at the indicated concentrations. NMDA receptor responses were activated by 10 μM glutamate (glut.) + 10 μM glycine (gly.), and data are normalized to the cells' initial responses to glutamate and glycine. A, solid bars represent the effect of mecamylamine on peak current amplitudes during the coapplication. Open bars represent the net charge calculated during the coapplication response. Hatched bars represent the peak amplitude of control glutamate/glycine responses after a 5-min wash. Data represent the average values for at least four cells under each condition with either S-(+)-mecamylamine (left) or R-(−)-mecamylamine (right). B, raw data from an experiment testing the effect of 100 μM S-(+)-mecamylamine and R-(−)-mecamylamine on NMDAR1 + NMDAR2b receptors. Note the large rebound currents occurring with the washout of mecamylamine, consistent with the relief of open channel block. In order to control for the intrinsic variability of the control responses, statistical analyses were based on t test comparisons between experimental data and repeated glutamate controls (i.e., sequential responses to the applications of 10 μM glutamate + 10 μM glycine). The peak amplitude of the repeated NMDA receptor controls were 0.958 ± 0.062 of the previous control responses, and the area of the repeated NMDA receptor controls were 1.042 ± 0.007 of the previous controls. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Unmarked bars are not significantly different from corresponding glutamate controls at the 0.05 level.

neuromuscular transmission, but the details regarding these differences were not described nor ever published.

The present findings indicate that while the various neuronal nAChR differ in their sensitivity to inhibition by mecamylamine and its stereoisomers, there seems to be little difference between S-(+)-mecamylamine and R-(−)-mecamylamine in terms of IC$_{50}$ values for a given receptor subtype. However, there appeared to be some significant differences in the off-rates for the mecamylamine isomers from the receptors. Specifically, S-(+)-mecamylamine appears to dissociate more slowly from $\alpha4\beta2$ and $\alpha3\beta4$ receptors than does R-(−)-mecamylamine.

Additionally, adult ($\alpha1\beta1\delta\epsilon$) muscle-type receptors appeared to be somewhat more sensitive to R-(−)-mecamylamine than to S-(+)-mecamylamine. This differential sensitivity of muscle receptors to the mecamylamine stereoisomers may have important clinical implications. For example, Tennant et al. (1984) found that "weakness" was one of the most intolerable side effects of mecamylamine when used as an aid to smoking cessation. In addition, in a recent double-blind placebo-controlled study investigating the safety of mecamylamine as a monotherapy for the treatment of Tourette's disorder, weakness was the most common adverse experience reported by patients treated with mecamylamine. Patients were asked each week of the 8-week

treatment study if they felt "fatigue or tiredness" (along with 113 other potential side effects). Twenty-eight percent (8 of 29) of patients in the mecamylamine group experienced this side effect versus 9% (3 of 32) in the placebo group (Silver et al., 2000). Taken together, the combination of an increased inhibitory effect on neuronal receptors and a decreased effect on muscle-type receptors, would suggest that in chronic (i.e., therapeutic) application, S-(+)-mecamylamine might be preferable to R-(−)-mecamylamine, especially in terms of equilibrium inactivation of neuronal receptors, and with decreased side effects associated with neuromuscular transmission.

Our competition experiments indicate that the mecamylamine stereoisomers are noncompetitive inhibitors of neuronal nAChR. This is consistent with our data that indicates that the inhibition produced by the mecamylamine isomers is voltage dependent, which is consistent with previous findings from this lab (Webster et al., 1999), as well as others (Ascher et al., 1979; Varanda et al., 1985; Fieber and Adams, 1991; Giniatullin et al., 2000).

Although the kinetics of recovery suggest a single exponential process for $\beta2$-containing receptors, the inhibition of $\alpha3\beta4$ receptors seems more complex. Recovery of $\alpha3\beta4$ receptors occurred in two phases, one phase of which was too slow to resolve over 30 min. This would suggest either multiple
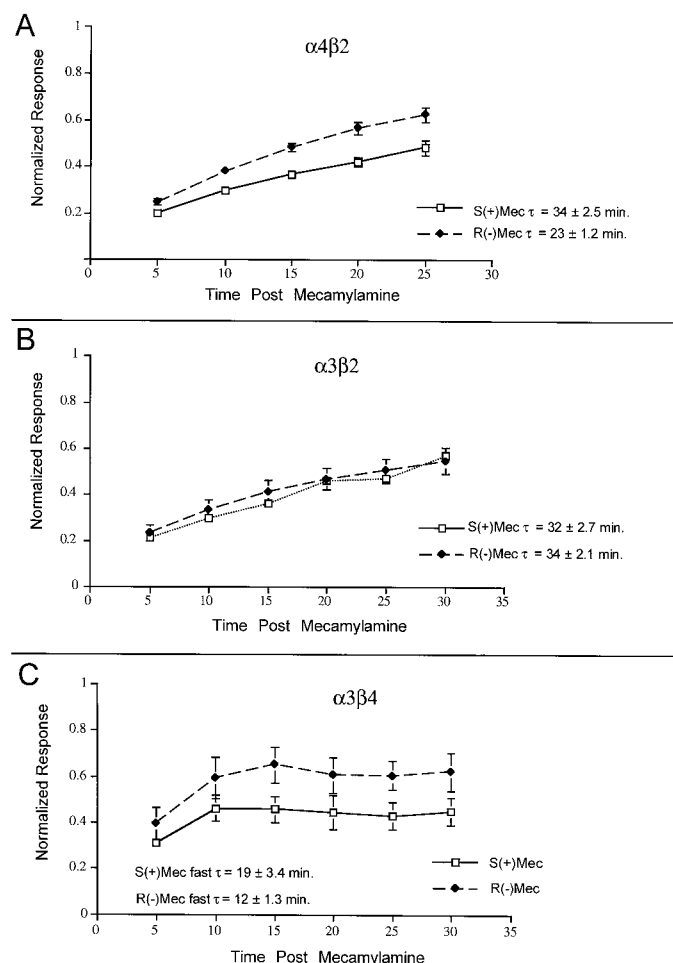
Fig. 6. The recovery of ACh control responses. Cells were monitored for their responses to control applications of ACh at 5-min intervals after 10 μM mecamylamine (Mec) was coapplied with ACh on: A, $\alpha4\beta2$ receptors; B, $\alpha3\beta2$ receptors; and C, $\alpha3\beta4$ receptors. Data from each oocyte were normalized to that cell's response to ACh alone. Each point represents the average normalized response of at least four cells.

sites of action or an inhibition-dependent allosteric conversion of receptors to a long-lived inactive state.

Our data provide an indication that S-(+)-mecamylamine may be a somewhat more desirable inhibitor of neuronal
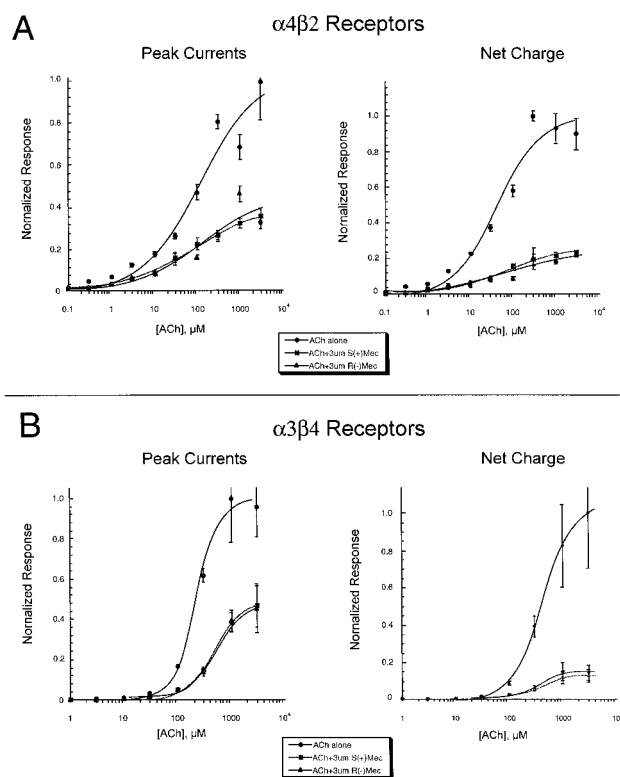
Fig. 7. Competition studies. A, ACh concentration response curves for $\alpha4\beta2$ receptors were determined with ACh alone or ACh coapplied with 3 μM S-(+)-mecamylamine or R-(−)-mecamylamine. Data were initially normalized to the 30 μM ACh responses obtained in the same cells and then scaled by the ratio of 30 μM ACh control responses to the maximal ACh responses, obtained with 3 mM ACh. Each point represents the average normalized response of at least four cells. Data in the left plot show the effect of mecamylamine (Mec) on peak currents. Data in the right plot show the effect of mecamylamine on the net charge during a coapplication of ACh and mecamylamine, normalized to the net charge in the responses to ACh alone. B, ACh concentration-response curves for $\alpha3\beta24$ receptors were determined with ACh alone or ACh coapplied with 3 μM S-(+)-mecamylamine or R-(−)-mecamylamine. Data were initially normalized to the 100 μM ACh responses obtained in the same cells and then scaled by the ratio of the 100 μM ACh control responses to the maximal ACh responses, obtained with 3 mM ACh. Each point represents the average normalized response of at least four cells. Data in the left plot show the effect of mecamylamine on peak currents. Data in the right plot show the effect of mecamylamine on the net charge during a coapplication of ACh and mecamylamine, normalized to the net charge in the responses to ACh alone.
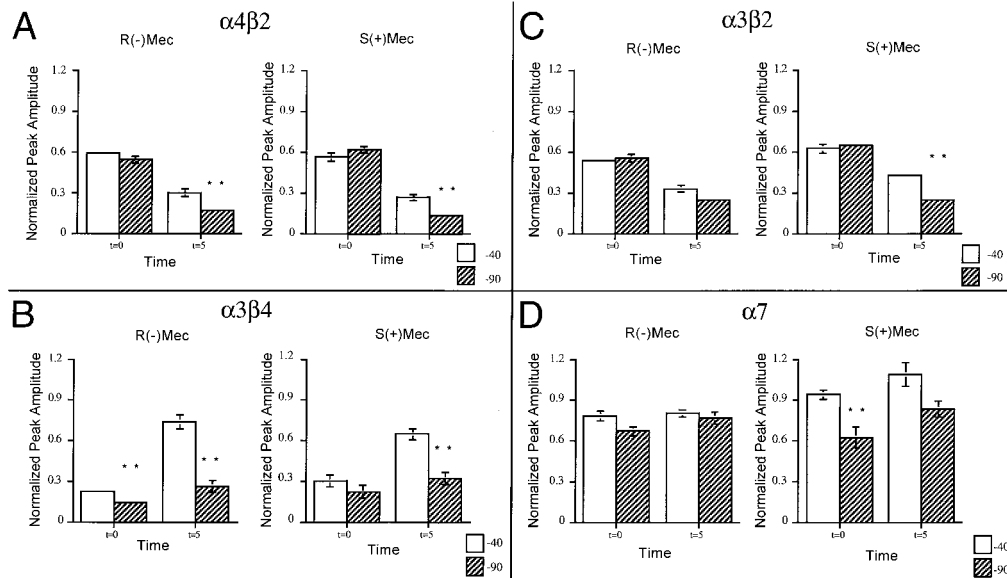
TABLE 2

Curve fits from competition experiments (Fig. 7)

	R_{max}	n	EC_{50}
			μM
$\alpha4\beta2$ peak currents			
ACh alone	1.0[a]	0.62–0.17	130–100
ACh + 3 μM S-(+)-mecamylamine	0.36–0.02	0.60–0.04	68–13
ACh + 3 μM R-(−)-mecamylamine	0.42–0.11	0.60–0.26	127–80
$\alpha4\beta2$ net charge			
ACh alone	1.0[a]	0.89–0.21	44–15
ACh + 3 μM S-(+)-mecamylamine	0.29–0.08	0.45–0.14	182–196
ACh + 3 μM R-(−)-mecamylamine	0.26–0.02	0.66–0.07	66–16
$\alpha3\beta4$ peak currents			
ACh alone	1.0[a]	2.03–0.22	230–14
ACh + 3 μM S-(+)-mecamylamine	0.47–0.02	1.92–0.22	456–38
ACh + 3 μM R-(−)-mecamylamine	0.49–0.02	1.65–0.11	478–28
$\alpha3\beta4$ net charge			
ACh alone	1.0[a]	1.57–0.04	423–10
ACh + 3 μM S-(+)-mecamylamine	0.15–0.01	2.06–0.62	343–54
ACh + 3 μM R-(−)-mecamylamine	0.13–0.01	1.40–0.25	378–68

[a] ACh R_{max} values defined as 1.0 so that the fit values for R_{max} in the presence of mecamylamine are expressed relative to ACh maximums.

Fig. 8. Voltage dependence of mecamylamine (Mec)-induced inhibition of peak currents. Mecamylamine was coapplied with agonist at either -40 or -90 mV. Inhibition was calculated relative to initial control ACh responses and measured during the coapplication ($t = 0$) or after a 5 min wash ($t = 5$). Concentrations of mecamylamine used were: A, 5 μM for $\alpha4\beta2$ receptors; B, 1 μM for $\alpha3\beta4$ receptors; C, 5 μM for $\alpha3\beta2$ receptors; and D, 10 μM for $\alpha7$ receptors. Each point represents measurements from at least four cells. Columns marked with a double asterisk were determined to be significantly different from the corresponding -40 mV control data at $p < 0.01$ by unpaired t tests.

nAChR than R-$(-)$-mecamylamine. However, a number of issues deserve further investigation. Recent in vivo studies suggest that mecamylamine has unique pharmacological effects at relatively low doses. For example, low (0.1 mg/kg s.c.), but not higher, doses of mecamylamine were found to attenuate the plasma corticosterone response to stress in rats (Newman et al., 2000) and improve executive cognitive function in aged primates (Terry et al., 1999). It would therefore

be of interest to look at the effects of mecamylamine and its stereoisomers on neuronal receptors containing the $\alpha5$ subunits, because these may better model the properties of some ganglionic and brain-type receptors (Conroy et al., 1992; Vernallis et al., 1993; Conroy and Berg, 1998). Additionally, other differences are likely to exist between the receptors expressed in oocytes and those found in vivo, such as additional complex subunit arrangements, post-translational
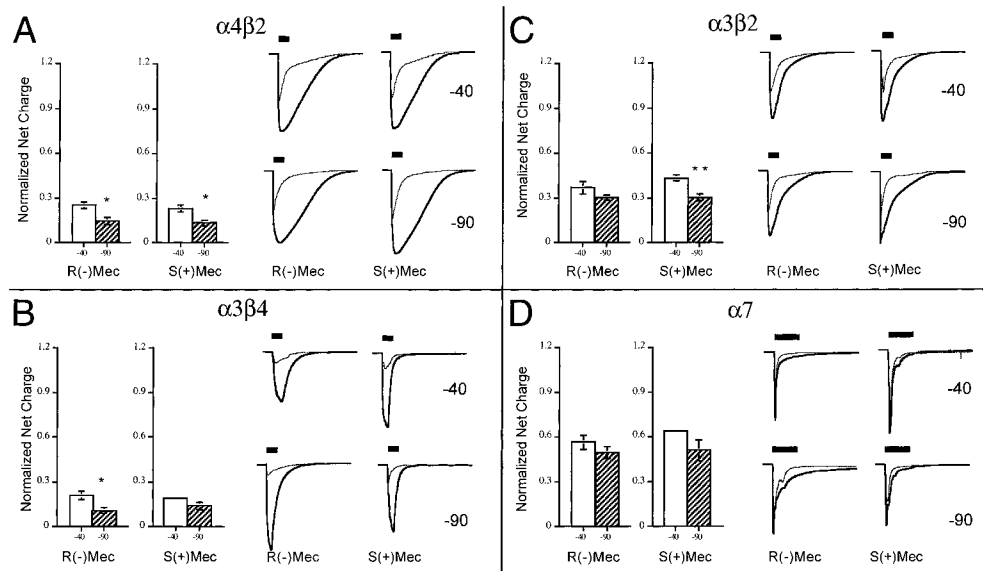

Fig. 9. Voltage dependence of mecamylamine (Mec)-induced inhibition of net charge during the coapplication of ACh and either S-$(+)$-mecamylamine or R-$(-)$-mecamylamine. Mecamylamine was coapplied with agonist at either -40 or -90 mV. Inhibition of net charge was calculated relative to the net charge in the initial control ACh responses. Representative traces are shown to the right of the bar graphs. Drug applications were 12 s in duration and correspond to the bars. Note that the rapidly desensitizing $\alpha7$ receptor-mediated responses are shown at an expanded time scale. In all cases, the response obtained in the presence of mecamylamine is shown as the thin line contained within the thick line that corresponds to the control ACh response obtained 5 min before the ACh/mecamylamine coapplication. The concentrations of mecamylamine used were: A, 5 μM for $\alpha4\beta2$ receptors; B, 1 μM for $\alpha3\beta4$ receptors; C, 5 μM for $\alpha3\beta2$ receptors; and D, 10 μM for $\alpha7$ receptors. Each point represents measurements from at least four cells. Analysis was conducted on the same responses that were represented in Fig. 8 in terms of peak amplitude. However, more significant effects of voltage were revealed when the data were analyzed in terms of net charge rather than peak currents. Columns marked with a double asterisk were determined to be significantly different from the corresponding -40 mV control data at $p < 0.01$ by unpaired t tests.

modifications, etc. These factors might also impact the sensitivity of native neuronal receptors to mecamylamine.

Although some previous reports have suggested that mecamylamine's effects in the brain may involve NMDA receptors (O'Dell and Christensen, 1988; McDonough and Shih, 1995), our data indicate that at least for the rat subtype consisting of NR1 and NR2b subunits, inhibitory effects of mecamylamine would be small and transient.

In conclusion, our results suggest that the new therapeutic potentials that have been proposed to exist for the central nervous system—active nicotinic antagonists may be better realized if we take advantage of the stereochemistry of mecamylamine to target more selectively the nicotinic receptors of the brain. Also, our studies of mecamylamine and its stereoisomers illustrate that there still may be much to learn from old drugs when we approach them with modern methodology and the refined perspective of molecular neurobiology.

References

Ascher P, Large WA and Rang HP (1979) Studies on the mechanism of action of acetylcholine antagonists on rat parasympathetic ganglion cells. *J Physiol (Lond)* **295:**139–170.

Baer JE, Paulson SF, Russo HF and Beyer KH (1956) Renal elimination of 3-methylaminoisocamphane hydrochloride (mecamylamine). *Am J Physiol* **186:**180–186.

Banerjee S, Punzi JS, Kreilick K and Abood LG (1990) [³H]mecamylamine binding to rat brain membranes. Studies with mecamylamine and nicotine analogues. *Biochem Pharmacol* **40:**2105–2110.

Bertrand D, Ballivet M and Rungger D (1990) Activation and blocking of neuronal nicotinic acetylcholine receptor reconstituted in *Xenopus* oocytes. *Proc Natl Acad Sci USA* **87:**1993–1997.

Blomqvist O, Ericson M, Engel JA and Soderpalm B (1997) Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine. *Eur J Pharmacol* **334:**149–156.

Blomqvist O, Ericson M, Johnson DH, Engel JA and Soderpalm B (1996) Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. *Eur J Pharmacol* **314:**257–267.

Chavez-Noriega LE, Crona JH, Washburn MS, Urrutia A, Elliott KJ and Johnson EC (1997) Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors h alpha2beta 2, h alpha2beta4, h alpha3beta2, h alpha3beta4, h alpha4beta2, h alpha4beta4 and h alpha7 expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther* **280:**346–356.

Conroy W and Berg D (1998) Nicotinic receptor subtypes in the developing chick brain: appearance of a species containing the $\alpha4$, $\beta2$ and $\alpha5$ gene products. *Mol Pharmacol* **53:**392–401.

Conroy WG, Vernallis AB and Berg DK (1992) The alpha 5 gene product assembles with multiple acetylcholine receptor subunits to form distinctive receptor subtypes in brain. *Neuron* **9:**679–691.

Elgoyhen AB, Johnson DS, Boulter J, Vetter DE and Heinemann S (1994) $\alpha9$: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* **79:**705–715.

Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF and Boulter J (2001) $\alpha10$: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. *Proc Natl Acad Sci USA* **98:**3501–3506.

Ericson M, Blomqvist O, Engel JA and Soderpalm B (1998) Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *Eur J Pharmacol* **358:**189–196.

Fieber LA and Adams DJ (1991) Acetylcholine-evoked curents in cultured neurones dissociated from rat parasympathetic cardiac ganglia. *J Physiol (Lond)* **434:**215–237.

Francis MM and Papke RL (1996) Muscle-type nicotinic acetylcholine receptor delta subunit determines sensitivity to noncompetitive inhibitors while gamma subunit regulates divalent permeability. *Neuropharmacology* **35:**1547–1556.

Frazier CJ, Buhler AV, Weiner JL and Dunwiddie TV (1998) Synaptic potentials mediated via α-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. *J Neurosci* **18:**8228–8235.

Giniatullin RA, Sokolova EM, Di Angelantonio S, Skorinkin A, Talantova MV and Nistri A (2000) Rapid relief of block by mecamylamine of neuronal nicotinic acetylcholine receptors of rat chromaffin cells in vitro: an electrophysiological and modeling study. *Mol Pharmacol* **58:**778–787.

Karler R, Calder LD and Bedingfield JB (1996) A novel nicotinic-cholinergic role in behavioral sensitization to amphetamine-induced stereotypy in mice. *Brain Res* **725:**192–198.

Martin BR, Martin TJ, Fan F and Damaj MI (1993) Central actions of nicotine antagonists. *Med Chem Res* **2:**564–577.

Martin BR, Onaivi ES and Martin TJ (1989) What is the nature of mecamylamine's antagonism of the central effects of nicotine? *Biochem Pharmacol* **38:**3391–3397.

McDonough JH Jr and Shih TM (1995) A study of the N-methyl-D-aspartate antagonistic properties of anticholinergic drugs. *Pharmacol Biochem Behav* **51:**249–253.

Mishina M, Takai T, Imoto K, Noda M, Takahashi T, Numa S, Methfessel C and Sakmann B (1986) Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature (Lond)* **321:**406–411.

Nadal R, Chappell AM and Samson HH (1998) Effects of nicotine and mecamylamine microinjections into the nucleus accumbens on ethanol and sucrose self-administration. *Alcohol Clin Exp Res* **22:**1190–1198.

Newman MB, Nazian S, Sanberg PR, Diamond D and Shytle RD (2001) Corticosterone-attenuating and anxiolytic properties of mecamylamine in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* **25:**609–620.

O'Dell TJ and Christensen BN (1988) Mecamylamine is a selective non-competitive antagonist of N-methyl-D-aspartate- and aspartate-induced currents in horizontal cells dissociated from the catfish retina. *Neurosci Lett* **94:**93–98.

Ozawa S, Kamiya H and Tsuzuki K (1998) Glutamate receptors in the mammalian CNS. *Prog Neurobiol* **54:**581–618.

Papke RL (1993) The kinetic properties of neuronal nicotinic receptors: genetic basis of functional diversity. *Prog Neurobiol* **41:**509–531.

Papke RL, Craig AG and Heinemann SF (1994) Inhibition of nicotinic acetylcholine receptors by BTMPS (Tinuvin 770), an additive to medical plastics. *J Pharmacol Exp Ther* **268:**718–726.

Papke RL and Thinschmidt JS (1998) The correction of alpha7 nicotinic acetylcholine receptor concentration-response relationships in *Xenopus* oocytes. *Neurosci Lett* **256:**163–166.

Reid MS, Mickalian JD, Delucchi KL and Berger SP (1999) A nicotine antagonist, mecamylamine, reduces cue-induced cocaine craving in cocaine-dependent subjects. *Neuropsychopharmacology* **20:**297–307.

Rose JE, Behm FM and Westman EC (1998) Nicotine-mecamylamine treatment for smoking cessation: the role of pre-cessation therapy. *Exp Clin Psychopharmacol* **6:**331–343.

Rose JE, Behm FM, Westman EC, Levin ED, Stein RM and Ripka GV (1994) Mecamylamine combined with nicotine skin patch facilitates smoking cessation beyond nicotine patch treatment alone. *Clin Pharmacol Ther* **56:**86–99.

Sanberg PR, Shytle RD and Silver AA (1998) Treatment of Tourette's syndrome with mecamylamine. *Lancet* **352:**705–706.

Schoenenberger B, Brossi A, George C and Flippen-Anderson J (1986) Preparation of optically active secondary amines by thermal decomposition of (methylbenzyl)urea analogs: absolute configuration of (+)- and (−)-mecamylamine. *Helv Chim Acta* **69:**283–287.

Silver AA, Shytle RD, Sheehan DV, Sheehan KH, Ramos A and Sanberg PR (2000) Safety and exploratory study of mecamylamine monotherapy for Tourette's syndrome. 2000 Nov 2–4; New Orleans: The 10th Annual Neuropharmacology Conference: Neuronal Nicotinic Receptors.

Stone CA, Torchiana ML, Meckelnberg KL, Stavorski J, Sletzinger M, Stein GA, Ruyle WV, Reinhold DF, Gaines WA, Arnold H and Pfisterk D (1962) Chemistry and structure-activity relationships of mecamylamine and derivatives. *J Med Pharm Chem* **91:**665–690.

Stone CA, Torchiana ML, Navarro A and Beyer KH (1956) Ganglionic blocking properties of 3-methylaminoisocamphane hydrochloride (mecamylamine): a secondary amine. *J Pharmacol* **117:**169–183.

Suchocki JA, May EL, Martin TJ, George C and Martin BR (1991) Synthesis of 2-exo- and 2-endo-mecamylamine analogues. Structure-activity relationships for nicotinic antagonism in the central nervous system. *J Med Chem* **34:**1003–1010.

Tennant FS Jr, Tarver AL and Rawson RA (1984) Clinical evaluation of mecamylamine for withdrawal from nicotine dependence. *NIDA Res Monogr* **49:**239–246.

Terry AV, Buccafusco JJ and Prendergast MA (1999) Dose-specific improvements in memory-related performance by rats and aged monkeys administered the nicotinic-cholinergic antagonist mecamylamine. *Drug Dev Res* **47:**127–136.

Varanda W, Aracava Y, Sherby SM, VanMeter WG, Eldefrawi ME and Albuquerque EX (1985) The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol Pharmacol* **28:**128–137.

Vernallis AB, Conroy WG and Berg DK (1993) Neurons assemble acetylcholine receptors with as many as three kinds of subunits while maintaining subunit segregation among receptor subtypes. *Neuron* **10:**451–464.

Webster JC, Francis MM, Porter JK, Robinson G, Stokes C, Horenstein B and Papke RL (1999) Antagonist activities of mecamylamine and nicotine show reciprocal dependence on beta subunit sequence in the second transmembrane domain. *Br J Pharmacol* **127:**1337–1348.

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