Biochemical Pharmacology, Vol. 40, No. 9, pp. 2105–2110, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

[^{3}H]MECAMYLAMINE BINDING TO RAT BRAIN MEMBRANES

STUDIES WITH MECAMYLAMINE AND NICOTINE ANALOGUES

SRABANI BANERJEE, JOHN S. PUNZI, KELLEY KREILICK and LEO G. ABOOD*
Department of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642, U.S.A.

(*Received* 11 *October* 1989; *accepted* 24 *May* 1990)

Abstract—Mecamylamine, an antagonist to nicotine, does not compete at the nicotinic recognition site, but is believed to block the ion channel of the nicotinic receptor. The present study demonstrates specific, saturable [^{3}H]mecamylamine binding in rat brain membranes. [^{3}H]Mecamylamine binding was destroyed by heating at 100° and trypsin. Scatchard analysis revealed the presence of two sites with K_d values of 9.6×10^{-8} and 1.1×10^{-6} M and B_{max} values of 7×10^{-12} and 3×10^{-11} mol/mg protein respectively. A good correlation was observed between the K_i values for [^{3}H]mecamylamine binding of a number of mecamylamine and related analogues and their ability to block nicotine-induced prostration in rats and seizures in mice. Inorganic cations, particularly divalent, and various ion channel blockers, such as phencyclidine and verapamil, exhibited a high affinity for the [^{3}H]mecamylamine site. Although mecamylamine did not block nicotine binding, nicotine and its analogues exhibited a high affinity for the [^{3}H]mecamylamine site, a finding which suggests that nicotine acts directly on ion channels as well as the nicotinic cholinergic recognition sites. The data are consistent with the notion that mecamylamine interacts with the open ion channel of the nicotinic receptor.

Mecamylamine (2-methylamino-2,3,3-trimethylnorbornane), which had originally been developed as a ganglionic blocking agent [1, 2], is believed to exert its pharmacologic action by antagonism at nicotinic cholinergic synapses in autonomic ganglia and the central nervous system [1–3]. Despite its widespread use in recent years as an antagonist to the peripheral and central effects of nicotine [4, 5], the site and molecular mechanism of its action are not understood. On the basis of receptor binding studies employing either [^{3}H]nicotine [6–8] or [^{3}H]methylcarbamylcholine, a specific nicotinic ligand [9], mecamylamine does not appear to interact with the nicotinic recognition site. From electrophysiologic studies it has been suggested that mecamylamine may be blocking ionic channels associated with the nicotinic recepter [10, 11]. Mecamylamine was shown to block acetylcholine-induced currents in crustacean muscle in a concentration- and voltage-dependent manner, and recovery of the blockade required the presence of the agonist [10].

The present study was undertaken to identify and characterize the binding site for mecamylamine in brain tissue with the use of [^{3}H]mecamylamine ([^{3}H]MEC) and to compare the affinity of mecamylamine and nicotine analogues.

Preliminary reports demonstrating specific [^{3}H]MEC binding to rat brain membranes have been published recently [12, 13].

* Correspondence: Dr. Leo G. Abood, Department of Pharmacology, University of Rochester, School of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY 14642.

METHODS

Preparation of brain subcellular fractions. Rat brains were homogenized in 15 vol. of 0.32 M sucrose with a loose fitting Potter–Elvehjem homogenizer, and after centrifuging the homogenate for 10 min at 1000 *g*, the supernatant was centrifuged at 20,000 *g* for 1 hr to yield the crude synaptosomal fraction, P_1. P_1 could be stored in 0.32 M sucrose at −70° for at least 1 month with little loss of activity. To obtain mitochondria and purer synaptosomal preparations, P_1 was layered onto a discontinuous gradient consisting of equal volumes of 0.8 M + 1.2 M sucrose and centrifuged at 100,000 *g* for 1 hr. After the mitochondria (bottom of tube), synaptosomes (intermediate) and myelin (top layer) were resuspended in 0.32 M sucrose, and centrifuged at 75,000 *g* for 1 hr, they were ready for use.

Preparation of rat brain membranes. With the exception of studies comparing [^{3}H]MEC binding in the various subcellular fractions of rat brain, all other studies were performed with rat brain membranes. Rat brains (Sprague–Dawley) were polytroned in 20 vol. of ice-cold water and centrifuged at 75,000 *g* for 1 hr. The pellet was washed twice by rehomogenization in 40 vol. of 40 mM $NaPO_4$ buffer, pH 7.4, and centrifuged at 20,000 *g* for 15 min. Th preparation, which could be stored in an ice bath for 1 week without loss of [^{3}H]MEC binding, was washed twice by homogenization in 10 mM Tris·HCl, pH 7.4, 0.32 M sucrose, and centrifugation at 20,000 *g* for 15 min.

[^{3}H]*Mecamylamine binding.* The incubation medium for [^{3}H]MEC binding consisted of 1 mL of the following (final concentration): 10 mM

Tris·HCl, pH 7.4, 0.32 M sucrose, 2×10^{-9} M [^{3}H]MEC (sp. act. 35 Ci/mmol), with or without various concentrations of unlabeled mecamylamine or other agents, plus 1 mg membrane protein. After incubation (in polypropylene test tubes) for 15 min in an ice-bath, the reaction was stopped by the addition of 2.5 mL of ice-cold 0.32 M sucrose. The contents were immediately filtered *in vacuo* using glass fiber GF/B filters, and after the filters were washed twice with 2.5 mL of 0.32 M sucrose, they were determined for radioactivity by liquid scintillation.

For Scatchard analysis the incubation medium consisted of 0.32 M sucrose with various concentrations of [^{3}H]MEC having a specific activity of 0.52 Ci/mmol. The lower specific activity was necessary since saturation of [^{3}H]MEC binding could not be achieved at higher levels of specific activity.

Pharmacological measurements. Mecamylamine and related agents were used for their ability to antagonize the nicotine-induced prostration syndrome as described elsewhere [6]. Briefly, the procedure involved the administration of 10 μL of a 5×10^{-3} M solution of nicotine·HCl. This dose of nicotine alone was the minimal dose in rats resulting in prostration of the hind and forelimbs. The results of the test agents were expressed as the ED_{50}, i.e. the dose required to block nicotine-induced prostration in at least the hindlimbs.

The various agents were also tested for their ability to prevent seizures following the administration of 2 mg/kg nicotine·HCl intraperitoneally to Swiss–Webster male mice weighing 20–30 g. A given dose of the test agent was administered 2 min prior to nicotine, and the minimal dose necessary to prevent nicotine-induced seizures was determined. A total of five mice were used for each agent tested, and the results are expressed as ED_{50}.

Materials. Mecamylamine, normecamylamine, dimethylaminoisocamphane, and *N*-(1,2,2)trimethyl-1-bicyclo[2.2.1.]-heptylbenzenamine were supplied by Merck Sharp & Dohme Research Laboratories. Pempidine, and other tetramethylpiperidines, amantadine, and exo-aminonorbornane were obtained from the Aldrich Chemical Co. Cocaine and phencyclidine were provided by the National Institute of Drug Abuse. The nicotine analogues were gifts of Philip Morris. [^{3}H]Mecamylamine was obtained from New England Nuclear.

RESULTS

Effects of various monovalent and divalent cations on [^{3}H]MEC binding. A number of monovalent and divalent inorganic cations were found to be inhibitory to [^{3}H]MEC binding at relatively low concentrations (Table 1). The concentration of NaCl and KCl needed to produce 50% inhibition of binding was 2 mM. The concentrations of the divalent cations Ca^{2+} and Mg^{2+} needed for 50% inhibition were 0.05 and 0.04 mM respectively. The Tris·HCl at a final concentration of 10 mM produced 50% inhibition and at 150 mM complete inhibition.

Effects of trypsin, heating and sodium dodecyl sulfate on [^{3}H]MEC binding. At a concentration of 0.1% sodium dodecyl sulfate [^{3}H]MEC binding was inhibited 70% (Table 1). Exposure of the membranes to 1 mg/mL of trypsin for 1 hr completely inhibited [^{3}H]MEC binding. Heating at 100° completely destroyed binding activity.

Scatchard analysis of [^{3}H]mecamylamine binding. The saturable binding curve for [^{3}H]MEC was obtained using [^{3}H]MEC with a specific activity of 0.52 Ci/mmol (Fig. 1). A Scatchard analysis of [^{3}H]mecamylamine binding, performed with [^{3}H]mecamylamine having a radioactive specific activity of 0.52 Ci/mmol, yielded a curvilinear plot with K_d values of 9.6×10^{-8} M and 1.1×10^{-6} M and B_{max} values of 7×10^{-12} mol/mg and 3×10^{-11} mol/mg protein respectively (Fig. 2).

Competition of various mecamylamine analogues and related agents for [^{3}H]mecamylamine binding. A number of mecamylamine analogues and related agents were tested for their ability to compete with [^{3}H]mecamylamine binding to rat brain membranes (Table 2). The most potent derivative was 3-dimethylaminoisocamphane with a K_i of 7.4×10^{-8} M. The *N*-benzyl derivative of isocamphane (BCB) and normecamylamine had K_i values of 2.5×10^{-6} M. In the norbornane series, exo-aminonorbornane had a K_i of 7.4×10^{-5} M, while BCB had a value of 2.5×10^{-6} M. Cyclohexylamine had a K_i of 4.7×10^{-5} M. Among the most effective

Table 1. Effects of various cations and treatments on [^{3}H]mecamylamine ([^{3}H]MEC) binding to rat brain membrane

Treatment	Conditions	[^{3}H]MEC binding	
		fmol/mg	% Binding
Boiling H_2O bath	1 hr	0	0
Trypsin, 1 mg/mL	1 hr	0	0
Sodium dodecyl sulfate 0.1%	1 hr	56	30
NaCl	2 mM	47	25
KCl	2 mM	47	25
$CaCl_2$	0.05 mM	47	25
$MgCl_2$	0.04 mM	47	25
Tris·HCl	10 mM*	93	50
	150 mM*	0	0
Sucrose (no Tris)		186	100

All assays were performed in 10 mM Tris·HCl + 0.32 M sucrose.
* Final concentration of Tris·HCl.

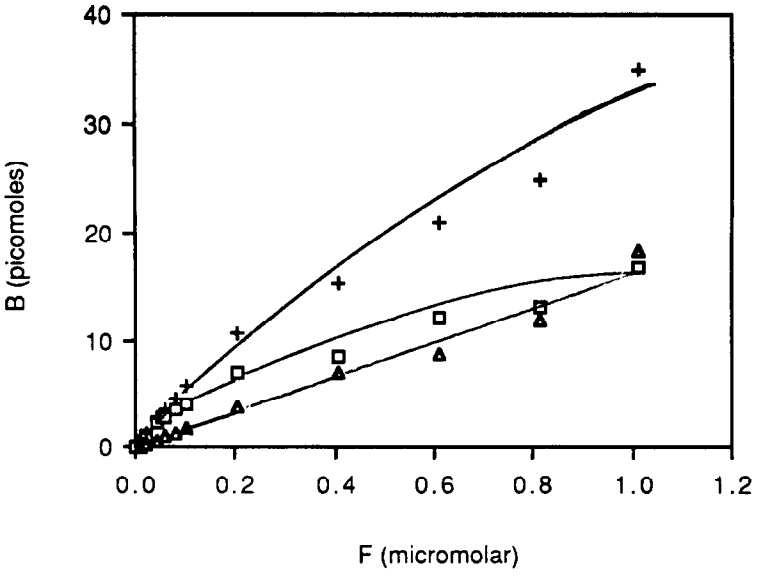

Fig. 1. Saturation analysis of [^{3}H]MEC binding to rat brain membranes. Membranes were incubated with increasing concentrations of [^{3}H]MEC in the presence (△—△) and absence (+—+) of 100 μM mecamylamine to obtain specific binding (□—□).

agents were phencyclidine (PCP) and pempidine which had K_i values of 5×10^{-6} M and 7.4×10^{-7} M respectively. Displacement curves for normecamylamine, tetramethylpiperidine, and pempidine are presented in Fig. 3.

Nicotine and its analogues were also found to compete for [^{3}H]mecamylamine binding; nicotine, *N'*-ethylnornicotine, 6-methylnicotine, and *N*-methylnicotine had K_i values of 6.3×10^{-7} M, 1.8×10^{-6} M, and 2×10^{-6} M respectively. Methylcarbamylcholine, a pure nicotine agonist, had a K_i value of 8×10^{-6} M.

The calcium channel blocker verapamil was an effective competitor for [^{3}H]mecamylamine binding with a K_i value of 7×10^{-7} M. Trihexyphenidyl, an antimuscarinic (M_2 type), had a K_i value of 4×10^{-7} M.

Antagonism of nicotine behavioral effect. The various agents were tested for their ability to prevent nicotine-induced prostration in rats following i.c.v. administration. The rank order of mecamylamine analogues and related agents agreed with their ability to compete for [^{3}H]mecamylamine binding (Table 2). Dimethylaminoisocamphane and mecamylamine were most effective with ED_{50} values of 15 and 25 nmol respectively; next were pempidine and tetra-

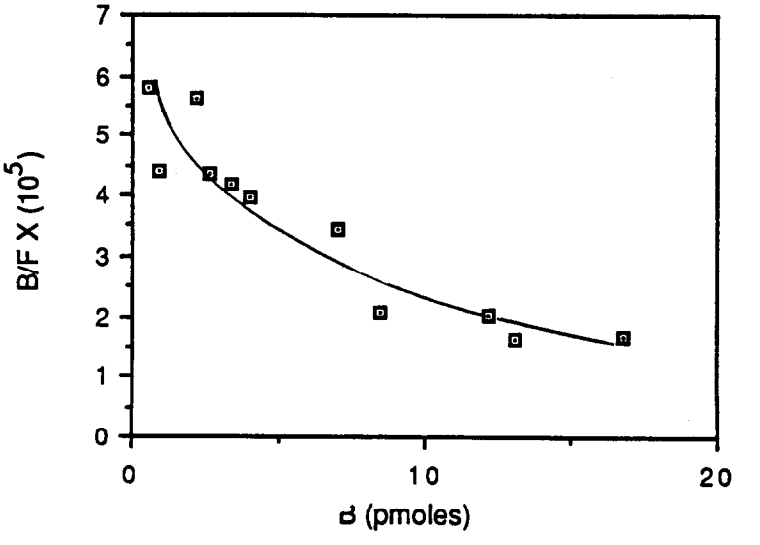

Fig. 2. Scatchard plot for [^{3}H]MEC binding to rat brain membranes.

Table 2. [^{3}H]Mecamylamine binding and pharmacologic activity of various mecamylamine analogues and related agents

Compound	[^{3}H]MEC binding K_i (M)	Prostration ED_{50} (nmol)	Seizures ED_{50} (mg/kg)
Mecamylamine	9.6×10^{-8}	25	10
Normecamylamine	2.6×10^{-6}	50	20
N-(1,2,2)Trimethyl-1-bicyclo-[2.2.1.]-heptylbenzenamine (BCB)	2.5×10^{-6}	70	40
Dimethylaminoisocamphane	7.4×10^{-8}	15	20
N'-Ethylnornicotine	6.3×10^{-7}	—	—
6-Methylnicotine	1.8×10^{-6}	—	—
N-Methylnicotine	2×10^{-6}	—	—
Methylcarbamylcholine	8×10^{-6}	—	—
exo-Aminonorbornane	7.4×10^{-5}	200	>50
Cyclohexylamine	4.7×10^{-5}	200	>50
Phencyclidine (PCP)	5×10^{-6}	75	>25*
Verapamil	7×10^{-7}	>50*	>20*
Pempidine	7.4×10^{-7}	40	10
2,2,6,6-Tetramethylpiperidine	1.8×10^{-6}	40	25
Piperidine phosphate	$>10^{-4}$	>200	Inact.
Trihexyphenidyl	4×10^{-7}	>200	Inact.
Atropine	2.5×10^{-6}	Inact.	Inact.
2,2,6,6-Tetramethyl-4-aminopiperidine	2×10^{-6}	50	30
Amantadine	1.3×10^{-5}	200	Inact.
Cocaine	9×10^{-7}	50	35

Agents were tested against nicotine-induced prostration, following i.c.v. injections in rats, and nicotine-induced seizures, following i.p. administration in mice. The ED_{50} values were based on five animals at each dose of agent, the standard error ranging within 10–15% of the mean. K_i values were determined from the formula: $K_i = IC_{50}/(1 + L/K_d)$, where L = [[^{3}H]MEC] = 2×10^{-9} M and $K_d = 1 \times 10^{-7}$ M.

* Drugs were lethal at this dose.

methylpiperidine with an ED_{50} of 40 nmol. Other effective antagonists were normecamylamine, tetramethylaminopiperidine and cocaine with values of 50 nmol and BCB and PCP, with values ranging from 70 to 75 nmol.

Ability of various agents to prevent nicotine-induced seizures. The various agents were tested for their ability to prevent nicotine-induced seizures, tremors, and loss of muscle body tone in mice. Among the mecamylamine analogues, the most

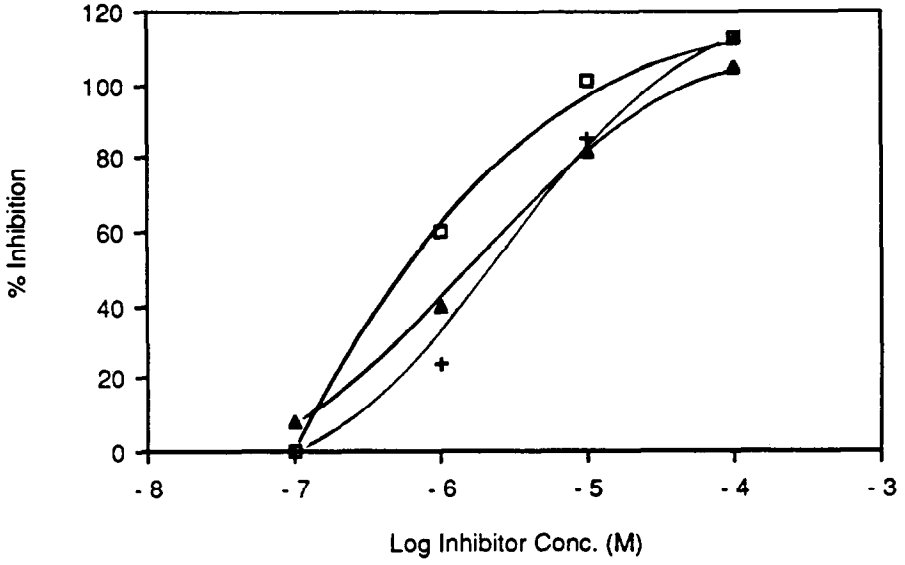

Fig. 3. Inhibition of [^{3}H]MEC binding by normecamylamine (+—+), tetramethylpiperidine (▲—▲) and pempidine (□—□), in rat brain membranes, at a [^{3}H]MEC concentration of 2×10^{-9} M. Specific binding in the absence of any inhibitor was 93 fmol/mg.

Table 3. [^{3}H]Mecamylamine binding in various rat tissues

Tissue	[^{3}H]MEC binding (fmol/mg)
Brain	93
Heart	63
Kidney	13
Liver	12

The values are an average of three separate experiments agreeing within 10–20%. The preparation of membranes for all tissues was as described in the text for brain.

effective was mecamylamine itself with an ED_{50} of 10 mg/kg, while normecamylamine and dimethylaminoisocamphane had values of 20 mg/kg. The relative effectiveness of various mecamylamine analogues in preventing nicotine-induced seizures was in agreement with that reported by Stone *et al.* [1]. Among the piperidine series, pempidine was comparable in antagonistic potency to mecamylamine; tetramethylpiperidine and tetramethylaminopiperidine had ED_{50} values of 25 and 30 mg/kg respectively. Other agents with antagonistic activity were BCB and cocaine with ED_{50} values of 40 and 35 mg/kg respectively. The remainder were either inactive or, like phencyclidine and verapamil, toxic at higher doses.

[^{3}H]*MEC binding to various rat tissues.* Among the various rat tissues, brain had the greatest density of [^{3}H]MEC binding sites, 93 fmol/mg membrane protein (Table 3). Whole heart contained 63, kidney 13, and liver 12 fmol/mg.

Subcellular distribution of [^{3}H]*MEC binding.* The subcellular distribution of [^{3}H]MEC binding revealed the greatest density of binding sites in the myelin fraction, with a value of 89 fmol/mg protein compared to 93 fmol/mg for whole rat brain membranes (Table 4). The density of sites in the synaptosomal fraction was 58 fmol/mg, while the mitochondria contained the least density with 31 fmol/mg.

DISCUSSION

The present study demonstrated the presence of specific, saturable [^{3}H]mecamylamine binding sites in rat brain membranes and also that the binding affinity of mecamylamine and related analogues correlated well with their pharmacologic activity. Similar to the findings of London and Majewska [13], Scatchard analysis revealed the presence of two sites. Among the isocamphane analogues, mecamylamine and dimethylaminoisocamphane had the highest affinity; the removal of both *N*-methyl substituents diminished activity. The binding site appears to be proteinaceous, since it was inhibited completely by trypsin and heating at 100°.

Table 4. [^{3}H]Mecamylamine binding in various subcellular fractions of rat brain

Subcellular fraction	[^{3}H]MEC binding (fmol/mg)
Whole rat brain membranes	93
Myelin	89
Synaptosomes	58
Mitochondria	31

All subcellular fractions were washed twice with 10 mM Tris·HCl, pH 7.4, 0.32 M sucrose as described in the text for whole rat brain membranes. The values are an average of three separate experiments agreeing within 8%.

Among the piperidine analogues, pempidine had the highest affinity and ability to block nicotine-induced seizures and prostration in rodents, whereas removal of the *N*-methyl group diminished activity and piperidine itself was inactive. Replacement of the *N*-methyl with a benzyl group diminished the potency of mecamylamine about 10-fold. Other heterocyclic amines tested, such as cyclohexylamine, aminonorbornane, and amantadine, possessed low affinity and were relatively ineffective as nicotine antagonists.

Although within the series of mecamylamine analogues and related heterocyclic amines, a good correlation was observed between competition for [^{3}H]MEC binding and antagonism to the pharmacologic effects of nicotine, some agents, such as the antimuscarinics, trihexyphenidyl and atropine, effectively competed for [^{3}H]MEC binding but were poor nicotine antagonists. One possible explanation for the anomaly is that only a subpopulation of [^{3}H]MEC binding sites comprise ionic channels of nicotinic receptors. Another possibility is that agents may bind to the ionic channels and, unlike mecamylamine, do not diminish the period of open channels [11]. Another agent with a high affinity for the MEC site but poor nicotine antagonism was verapamil, a calcium-channel blocker. One difficulty in screening verapamil for nicotine antagonism was its toxicity at lower doses.

The fact that nicotine and its analogues exhibited an affinity for the mecamylamine binding site raises the possibility that nicotine may be affecting the ionic channel associated with the nicotine cholinergic receptor. Mecamylamine does not bind to the nicotinic recognition site, so that its antagonism to nicotine does not involve the recognition site [7–9, 11]. The functional significance of this finding is not clear, insofar as mecamylamine is antagonistic to the pharmacologic effects of nicotine. One possibility is that nicotine, unlike mecamylamine which diminishes the period of open channels [11], may be prolonging the period, i.e. acting as an inverse agonist at the mecamylamine recognition site on the ionic channel.

Evidence that mecamylamine and pempidine are ion channel blockers derives from voltage-clamp studies with cat fish retinal cones demonstrating that both agents non-competitively block the excitatory response to *N*-methyl-*d*-aspartate and that the blockade is highly voltage sensitive [14]. In another study [11], mecamylamine noncompetitively blocked indirect muscle twitches in frog sartorius muscle with no effect on membrane potential, overshoot, or the action potential. It also produced a voltage- and concentration-dependent depression of the peak amplitude of endplate currents and diminished the time period of open channels. With the use of [^{3}H]perhydrohistrionicotoxin, a ligand to probe the ionic channel of the nicotinic cholinergic receptor in the electric organ of *Torpedo ocellata*, Eldefrawi *et*

al. [15] demonstrated that nicotine interacts with the ionic channel. At lower concentrations nicotine acts as an agonist by enhancing [^{3}H]perhydrohistrionicotoxin binding, whereas at higher concentrations it acts as an antagonist by inhibiting binding. It has been reported [13] that nicotine enhances [^{3}H]MEC binding to rat brain membranes following brief exposure to nicotine, but not during equilibrium. In the present study, nicotine was inhibitory to [^{3}H]mecamylamine binding to brain membranes at all concentrations, a finding which suggests that it was acting primarily as an antagonist. Another nicotinic ganglionic blocking agent, chlorisondamine, was also shown to block the open channel of the frog neuromuscular cholinergic junction [16]. Amantadine, which has also been demonstrated to block the nicotinic cholinergic ion channel [17], was also found to compete effectively for [^{3}H]MEC binding and to antagonize the behavioral effects of nicotine.

The presence of [^{3}H]MEC binding in mitochondria as well as nerve endings and myelin-axonal subcellular organelles suggests that the MEC sites are not necessarily associated with voltage-gated ion channels. Although excitable tissues such as brain and heart contain the preponderance of MEC binding sites, some specific MEC binding was observed in liver and kidney. Potassium selective channels exhibiting inward rectification, but which are not voltage- or calcium-dependent, have been demonstrated in hepatocytes using the patch-clamp technique [18].

Acknowledgements—We would like to thank Xin Lu for her assistance in the behavioral studies. This work was supported by H.E.W. Grant DA-00464. J. S. P. was supported by Postdoctoral Training Grant DA-07232.

REFERENCES

1. Stone CA, Torchiana ML, Navarro A and Beyer KH, Ganglionic blocking properties of 3-methylaminoisocamphane hydrochloride (mecamylamine): A secondary amine. *J Pharmacol Exp Ther* **117**: 169–183, 1956.
2. Stone CA, Torchiana ML, Meckelnberg KL and Stavorski J, Chemistry and structure–activity relationships of mecamylamine and derivatives. *J Med Pharm Chem* **5**: 665–686, 1962.
3. Volle RL and Koelle GB, Ganglionic stimulating and blocking agents. In: *The Pharmacological Basis of Therapeutics* (Eds. Goodman LS and Gilman A), pp. 565–574. Macmillan, New York, 1975.
4. Stolerman IP, Psychopharmacology of nicotine: Stimulus effects and receptor mechanisms. In: *Handbook of Psychopharmacology* (Eds. Iversen LL, Iversen SD and Snyder SH), Vol. 19, pp. 421–465. Plenum Press, New York, 1987.
5. Henningfield JE, Miyasato K, Johnson RE and Jasinski DR, Rapid physiologic effects of nicotine in humans and selective blockade of behavioral effects by mecamylamine. *Problems of Drug Dependence*, NIDA Monograph No. 43, pp. 259–265. NIDA, Washington, DC, 1983.
6. Abood LG, Reynolds DT, Booth H and Bidlack JM, Sites and mechanisms of nicotine's action in rat brain. *Neurosci Biobehav Rev* **5**: 479–486, 1981.
7. Marks MJ and Collins AC, Characterization of nicotine binding in mouse brain and comparison with the binding of α-bungarotoxin and quinuclidinyl benzilate. *Mol Pharmacol* **22**: 554–564, 1982.
8. Abood LG, Grassi S and Constanza M, Binding of optically pure (−)-[^{3}H]nicotine to rat brain membranes. *FEBS Lett* **157**: 147–149, 1983.
9. Abood LG and Grassi S, [^{3}H]Methylcarbamylcholine: A new radioligand for studying brain nicotine receptors. *Biochem Pharmacol* **35**: 4199–4202, 1986.
10. Lingle C, Blockade of cholinergic channels by chlorisondamine on a crustacean muscle. *J Physiol (Lond)* **339**: 395–417, 1983.
11. Varanda WA, Aracava Y, Sherby SM, Van Meter WG, Eldefrawi ME and Albuquerque EX, The ACh receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. *Mol Pharmacol* **28**: 128–137, 1985.
12. Banerjee S, Punzi JS and Abood LG, [^{3}H]Mecamylamine binding and the brain nicotinic receptor. *Soc Neurosci Abstr* **15**: 1194, 1989.
13. London ED and Majewska MD, Binding of [^{3}H]mecamylamine to the nicotinic receptor complex in the rat brain: Modulation by ATP. *Soc Neurosci Abstr* **14**: 64, 1989.
14. O'Dell TJ and Christensen BN, Mecamylamine is a selective noncompetitive antagonist of NMDA and ASP-induced currents in horizontal cells dissociated from the catfish retina. *Neurosci Lett* **94**: 93–98, 1988.
15. Eldefrawi AT, Miller ER and Eldefrawi ME, Binding of depolarizing drugs to the ionic channels of the nicotine acetylcholine receptor. *Biochem Pharmacol* **31**: 1819–1822, 1982.
16. Neely A and Lingle CJ, Trapping of an open channel blocker at the frog neuromuscular acetylcholine channel. *Biophys J* **50**: 981–986, 1986.
17. Warnick JE, Maleque MA and Albuquerque EX, Interaction of bicyclo-octane analogs of amantadine with ionic channels of the nicotinic acetylcholine receptor and electrically excitable membrane. *J Pharmacol Exp Ther* **228**: 73–79, 1984.
18. Henderson RM, Graf J and Boyer JL, Inward-rectifying potassium channels in rat hepatocytes. *Am J Physiol* **256**: G1028–G1035, 1989.