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3-[2,4-Dimethoxybenzylidene]anabaseine (DMXB) selectively activates rat $\alpha 7$ receptors and improves memory-related behaviors in a mecamylamine-sensitive manner

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Abstract

The $\alpha 7$ nicotinic receptor agonist 3-[2,4-dimethoxybenzylidene]anabaseine (DMXB; GTS-21) was investigated for its ability to: (1) activate a variety of nicotinic receptor subtypes in *Xenopus* oocytes; (2) improve passive avoidance and spatial Morris water task performances in mecamylamine-sensitive manners in bilaterally nucleus basalis lesioned rats; and (3) elevate high-affinity [^{3}H]acetylcholine (ACh) and high-affinity α-[^{125}I]bungarotoxin binding in rat neocortex following 2 weeks of daily injections. DMXB (100 μM) activated $\alpha 7$ homo-oligomeric receptors, without significant activity at $\alpha 2$-, $\alpha 3$- and $\alpha 4$-containing subtypes. Mecamylamine blocked rat $\alpha 7$ receptors weakly if co-administered with agonist, but much more potently when pre-applied. Bilateral ibotenic acid lesions of the nucleus basalis interfered with passive avoidance and spatial memory-related behaviors. DMXB (0.5 mg/kg, i.p.) improved passive avoidance behavior in lesioned animals in a mecamylamine-sensitive manner. DMXB (0.5 mg/kg 15 min before each session) also improved performance in the training and probe components of the Morris water task. DMXB-induced improvement in the probe component but not the training phase was mecamylamine-sensitive. [^{3}H]ACh binding was elevated after 14 days of daily i.p. injections with 0.2 mg/kg nicotine but not after 1 mg/kg DMXB. Neither drug elevated high-affinity α-[^{125}I]bungararotoxin binding over this interval. © 1997 Elsevier Science B.V.

Keywords: Morris water task; Nicotinic receptor; Passive avoidance; *Xenopus* oocyte

1. Introduction

Nicotine has been studied as a potential treatment for Alzheimer's disease because of its ability to improve a variety of spatial memory and non-spatial avoidance tasks in animals and to enhance delayed recall, attention and other memory-related behaviors in humans [12,14,28]. While the results of initial studies with nicotine in Alzheimer's disease have been promising [20,26], they have been limited by the many undesirable actions of this drug at doses only slightly higher than those improving performance. However, with the recent discovery of multiple nicotinic receptor subtypes in brain (e.g., $\alpha 2$–4, $\alpha 7$; $\beta 2$–4), it may now be possible to develop nicotinic agents with more selective activity on those receptor subtypes associated with learning- and memory-related behaviors [9,23].

$\alpha 7$ nicotinic receptors are among those hypothesized to be involved in learning and memory because of their high density in neocortex and hippocampus, regions associated with these behaviors [1,3,5,15,27]. These depolarizing receptors are also notable for their high permeability to calcium ions, comparable to that of NMDA R1 receptors [5,27]. Recently, the novel 3-substituted benzylideneanabaseine compound, 3-[2,4-dimethoxybenzilidene]anabaseine (DMXB, also referred to as GTS-21 in some studies), was found to possess $\alpha 7$ agonist activity and to improve passive and active avoidance behaviors in nucleus basalis lesioned rats [18]. It also improved eye-blink avoidance behavior in senescent rabbits at similar doses [29]. DMXB was at least partially selective for $\alpha 7$ receptors, with no agonist activity at $\alpha 4\beta 2$ nicotinic receptors, based on studies with *Xenopus*

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oocytes [13]. Based on these and related studies, DMXB recently began clinical trials for potential therapeutic activity in Alzheimer's disease.

However, important questions about the biological activity of DMXB remain unanswered. For example, its potential agonist activity at other nicotinic receptor subtypes has not been characterized, nor has the nicotinic nature of its activity in passive avoidance behavior been demonstrated with a brain-penetrant receptor-antagonist such as mecamylamine. Further, spatial memory-related behavior has been studied only marginally, particularly with respect to mecamylamine sensitivity. Spatial learning and memory appear to involve at least some neuronal pathways distinct from those underlying avoidance behaviors [22], and the relative importance of $\alpha 7$ receptor activity in each has not been studied. We therefore investigated whether DMXB: (1) activated a variety of nicotinic receptor subtypes that had not been evaluated previously in *Xenopus* oocytes; (2) improved acquisition and retention in the Morris water task; and (3) affected spatial and avoidance behaviors in a mecamylamine-sensitive manner. The nucleus basalis-lesioned rat was chosen for behavioral studies as a model for cholinergic and memory-related hypofunction, based on earlier results demonstrating that nicotinic agonists counteract behavioral deficits in this system [18]. Since mecamylamine is reportedly a relatively weak antagonist at $\alpha 7$ receptors when co-applied with agonists in the chick [5], we also investigated its potency at these receptors in rat under conditions more analogous to our behavioral study, i.e. pre-exposure to the antagonist prior to agonist. It was hypothesized that mecamylamine would be more potent under this condition because of the non-competitive nature of the mecamylamine antagonism as well as the tendency for the receptor to de-sensitize so quickly.

Another part of this study pertained to the possible role that $\alpha 4\beta 2$ receptors may play in vivo with respect to the behavioral actions of DMXB. Activation of $\alpha 4\beta 2$ nicotinic receptors, which are the predominant subtype other than $\alpha 7$ in brain based on binding studies, has also been associated with memory-related improvement in rodents [2,6,11,21]. Although DMXB has no direct agonist activity at these receptors, it is conceivable that it may activate them indirectly in vivo, either through a polysynaptic process triggered by $\alpha 7$ receptors or through DMXB-metabolite formation. $\alpha 4\beta 2$ agonists such as nicotine typically increase the density of these receptors over time in brain (so-called up-regulation), as measured with high-affinity ligand binding [11]. We therefore investigated whether chronic administration of DMXB up-regulated $\alpha 4\beta 2$ receptor-density as measured with high-affinity [^{3}H]acetylcholine (ACh) binding in a manner similar to that of nicotine administration. α-Bungarotoxin binding was measured in the same samples to determine whether $\alpha 7$ receptors were up-regulated by chronic, selective activation of these receptors with DMXB as well.

2. Materials and methods

2.1. Chemicals and solutions

Chemicals and drugs were obtained from Sigma Chemicals (St. Louis, MO) unless otherwise specified. DMXB was synthesized as described previously and generously provided by John Zoltewicz of the University of Florida [30].

2.2. Animals

Male Sprague-Dawley albino rats (250–350 g) were purchased from Charles River Laboratories (Boston, MA) and maintained in the University of Florida Health Center Vivarium according to NIH guidelines, on a 12:12 h day/night cycle. They had ad libitum access to food (Purina rat chow) and water. Female *Xenopus* were housed in aquarium tanks maintained precisely at 18°C to reduce potential problems with seasonal variability in oocyte viability. Frogs were fed frog brittle (Nasco) and kept on a 12:12 h light/dark cycle.

2.3. Oocyte recordings

Oocytes were prepared and recordings made as described previously [7]. Frogs were anesthetized by submersion in 0.1% (3)-aminobenzoic acid ethyl ester and several lobes of the ovary surgically removed through a small incision in the abdomen wall. Oocytes were freed from the follicle cells by treatment with collagenase (in calcium-free Barth's solution: 88 mM NaCl, 1 mM KCl, 15 mM HEPES (pH 7.6), 0.33 mM $MgSO_4$, 0.1 mg/ml gentamicin sulfate) for 2 h at room temperature and Stage 5 oocytes were isolated. Oocytes were rinsed and stored at 18°C in Barth's saline (88 mM NaCl, 1 mM KCl, 15 mM HEPES (pH 7.6), 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 0.1 mg/ml gentamicin sulfate) before and after microinjection with RNA. Microinjection was conducted with a Drummond Scientific 'Nanoject Variable' automatic injector. Oocytes were injected with a 50 nl solution of rat $\alpha 7$ mRNA (2–10 mg/ml; derived from the cDNA containing HIP 306 plasmid kindly provided by Dr. Jim Boulter, Salk Institute) and incubated 2–7 days at 18°C before electrophysiological recording.

For electrophysiological recordings, oocytes were perfused in a Warner Instruments RC-8 recording chamber with a perfusion solution containing 115 mM NaCl, 2.5 mM KCl, 10 mM HEPES (pH 7.2), 1.8 mM $CaCl_2$ and 1 μM atropine to block muscarinic responses. Perfusion was continuous at a rate of 10 ml/min. Drugs were diluted in perfusion solution and applied using a solenoid valve to switch from perfusion to drug solutions. Current responses to drug administration were studied under two electrode voltage clamps at a holding potential of -70 mV using a Dagan Corp. TEV-200 voltage clamp connected to a 386-

SX IBM computer using a TL-1 DMA interface (Axon Instruments). Micropipettes were filled with 3 M KCl and had resistances of 0.5–2 MΩ. Drug responses are analyzed with PClamp software (Axon Instruments). Oocytes with resting potentials of less than −30 mV were rejected.

2.4. High-affinity [^{3}H]ACh binding and α-[^{125}I]bungarotoxin binding

Rats were decapitated, and cerebral cortices were removed and homogenized in 10 vol of ice-cold Krebs-Ringer's HEPES (KRH) buffer (NaCl, 118 mM; KCl, 4.8 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; and HEPES, 20 mM; pH adjusted to 7.5 with NaOH), and then assayed for high-affinity [^{3}H]ACh or α-[^{125}I]bungarotoxin binding as described previously [18]. Atropine (1 μM) and physostigmine (10 μM) were added to prevent muscarinic receptor binding and ACh-hydrolysis, respectively. Binding assays were conducted at 4°C in KRH buffer. The final incubation contained 500–800 μg protein/250 μl with 4 nM [^{3}H]ACh or 0.5 nM α-[^{125}I]bungarotoxin. Binding was terminated by diluting with 3 ml of ice-cold KRH buffer, followed immediately by filtration through glass fiber filters soaked in buffer containing 0.5% polyethylenimine. The filters were washed four times with 3-ml aliquots of ice-cold buffer. Nonspecific binding was determined with 10 μM or 100 μM unlabeled nicotine for ACh and α-bungarotoxin binding, respectively.

2.5. Behaviors

Passive avoidance behavior was measured in nucleus basalis lesioned rats after intraperitoneal (i.p.) injections of DMXB. For lesions, male 5-month-old Sprague-Dawley rats were anesthetized with 50 mg/kg sodium pentobarbital (i.p.) and then infused bilaterally with 1 μl of 5 μg/μl ibotenic acid in phosphate-buffered saline, pH 7.4, into the nucleus basalis as described previously [17]. Infusion coordinates were anterior 7.0 mm, lateral 2.6 mm and vertical 6.5 mm according to Paxinos and Watson [24]. Following surgery, animals were returned to their individual home cages and fed semi-solid mash made from Purina rat chow for several days. One month later, animals were trained in a two-chamber passive avoidance paradigm. Animals received two i.p. injections on contralateral sides, 5 min apart, according to the following protocol: saline–saline (control); saline–DMXB (DMXB); saline–mecamylamine (mecamylamine); or mecamylamine–DMXB (both). This paradigm permitted pre-exposure to antagonist before agonist. Fifteen min after the second injection, animals were placed in the lit compartment, and allowed to enter the dark adjoining chamber. Each animal entered the dark chamber within the 5-min cutoff training interval. Those animals that entered the second chamber received a mild foot shock (0.8 mA) for 1 s. Rats were tested for latency 24 h later for up to 5 min; this test also began 15 min after injection. Statistical analyses utilized rank order nonparametric comparisons of latencies [10].

Morris water task behavior was investigated in lesioned animals according to the procedure of Morris [19] as modified by Paylor and Rudy [25]. Animals received 3 days of 12 training sessions/day beginning in the morning of each day. 24 h after the last training session, animals received a probe-trial session in which the platform was removed from the circular tub (110 cm diameter; 60 cm height) using opaque, milk-colored water (28°C). Visual cues were maintained constant during each training and testing interval, including the trainer. During training, escape latencies were measured for each session and the slope of the learning curve determined for each animal. These slopes were combined for each group and compared among groups using the rank-order statistic [10]. Rank sum analyses were used because of the possibility that lesioning resulted in a non-normal population compared to lesioned animals, which would render parametric tests incomprehensible. Animals received i.p. drug injections 15 min before the beginning of the first training interval each day, and 15 min before the probe-trial.

During training, animals were hand-guided to the platform if they did not find it by 60 s; these training intervals were given a 60 s value. The time-to-training curve was determined for each animal, and the areas under the curve were separated between the first 24 and the last 12 training sessions in order to compare groups during the early vs. later stages of training. Comparisons among groups for this statistic used the non-parametric rank order statistic [10]. Swimming distance was measured for the entire 36 trials for each animal and compared using one way ANOVA. During the probe-trial, swimming distance and percent time in target vs. non-target quadrants were determined over a 60-s interval; one-way ANOVA was used for time spent in each quadrant and swimming distance. Paths swam by animals were video recorded and traced onto transparencies. Total distances for the probe-trials were measured by a plenimeter. Parametric analyses (one-way ANOVA) were used for time spent in each quadrant and swimming distance.

Histological assessments of the ibotenic acid placements in the nucleus basalis were made after behavioral measurements, using cholinesterase staining in formalin-fixed tissues [17]. These injections reduced the number of cholinesterase-staining cells by over 85% in the nucleus basalis [17], with some loss of staining in the globus pallidus and thalamus as well, as determined with the NIH Image 1.47 program [16].

3. Results

ACh displayed typical agonist activity at each nicotinic receptor subtype combination expressed in *Xenopus* oocytes (EC_{50} concentration used for normalization de-

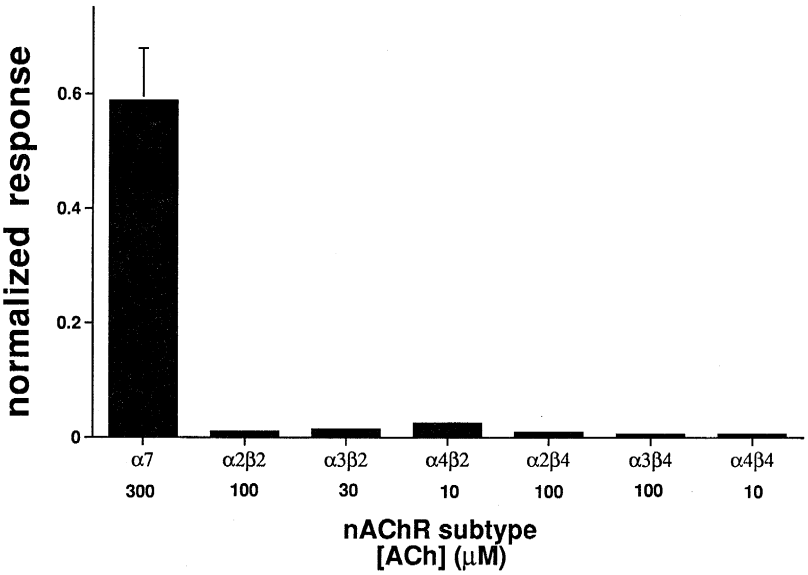

Fig. 1. Responses of nicotinic receptor subtypes to DMXB in *Xenopus* oocytes. Whole cell current responses to 100 μM DMXB were measured in oocytes expressing specific nicotinic acetylcholine receptor subtypes as described in the text. Responses to DMXB were expressed as a fraction of the response of the same oocyte to an EC_{50} concentration of ACh measured 5 min prior to DMXB application. ACh concentrations (μM) used for each receptor subtype are shown below each receptor subtype ($n = 3–4$/group).

scribed in Fig. 1). DMXB was an agonist at $\alpha 7$ homo-oligomeric receptors but at no other subtypes tested in this system (subtypes formed by the pairwise injection of $\alpha 2$, $\alpha 3$ and $\alpha 4$ subunits with either $\beta 2$ or $\beta 4$. At no subtype other than $\alpha 7$ was even 3% of the EC_{50} ACh concentration observed. The 100 μM DMXB concentration was selected based on earlier studies demonstrating it to be almost fully efficacious at $\alpha 7$ receptors in this oocyte system [7].

Prior to using mecamylamine in behavioral studies, its potency at rat $\alpha 7$ receptors was investigated (Fig. 2). While relatively weak when co-applied with 300 μM ACh (IC50 of 19 μM), the potency of this non-competitive channel blocker was increased about 10-fold by pre-exposure to the receptor before addition of agonist (Fig. 2). Therefore, for all behavioral studies, mecamylamine was injected 5 min prior to DMXB when both were administered.

Bilateral injections of ibotenic acid into the nucleus basalis interfered with passive avoidance (Fig. 3) and

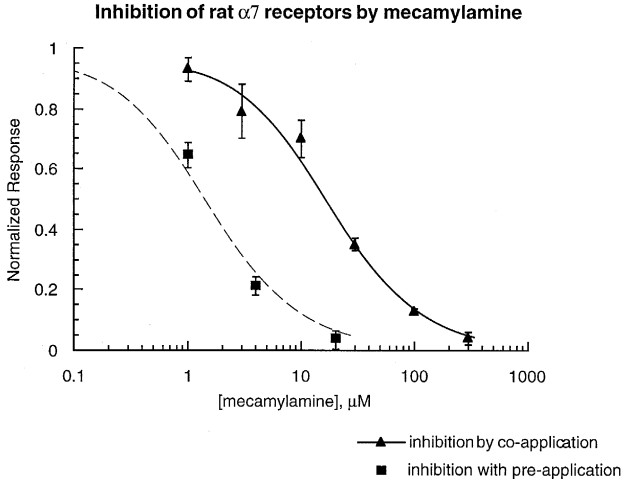

Fig. 2. Inhibition of rat $\alpha 7$ receptor responses to 300 μM ACh by mecamylamine. Triangles represent the inhibition oobtained when mecamylamine is co-applied with ACh; solid curve is fit of data, giving IC_{50} of 19 μM. Squares represent results of pre-exposure to mecamylamine prior to co-application of mecamylamine and ACh; dotted line is curve fit for these results. Note shift by 1 log unit to an IC_{50} of approximately 2 μM.

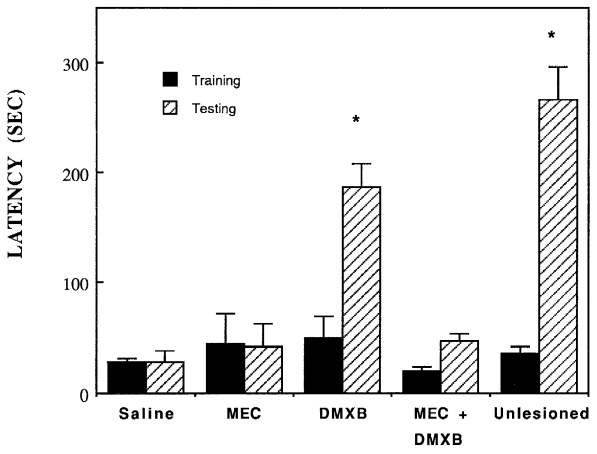

Fig. 3. Effects of mecamylamine on DMXB-induced improvement in passive avoidance behavior. Adult Sprague-Dawley albino rats were bilaterally nucleus basalis-lesioned and tested for passive avoidance behavior 1 month later as described in the text. Latencies for training and testing intervals were determined 15 min after i.p. injection of saline vehicle, 0.5 mg/kg DMXB, 0.2 mg/kg mecamylamine (MEC) or both drugs, and described as mean ± S.E.M. of five animals/group. * $P < 0.05$ compared to saline-injected group, one-way ANOVA.

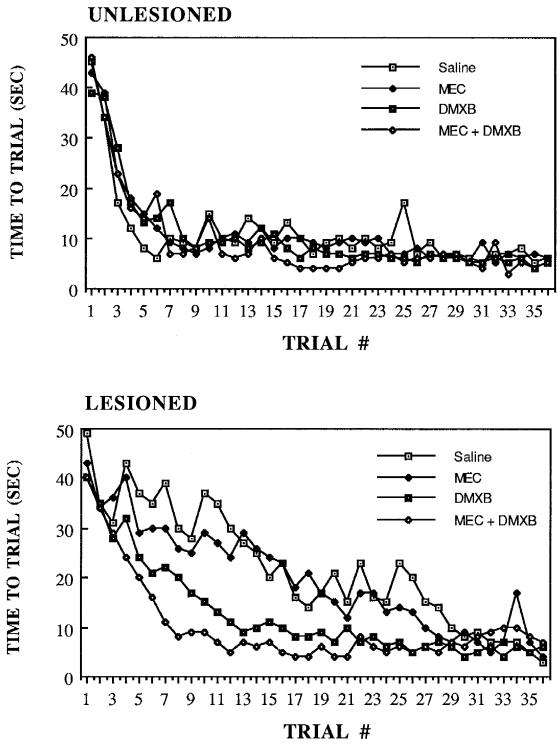

Fig. 4. Effects of DMXB on training in the Morris water task in bilaterally nucleus basalis-lesioned rats. Animals that were lesioned or sham-operated began training 1 month later as described in the text. Saline diluent, 0.5 mg/kg DMXB, 0.2 mg/kg mecamylamine (MEC), or both drugs were injected i.p. 15 min prior to the first trial each day in the lesioned and unlesioned groups. Values are the mean times necessary to find the target (maximum: 60 s) ($n = 5$). Intergroup comparisons are described in Table 1.

Morris water task behaviors (Fig. 4 and Fig. 5). DMXB (0.5 mg/kg i.p.) improved the performance of lesioned animals in a mecamylamine-sensitive (0.2 mg/kg i.p.)

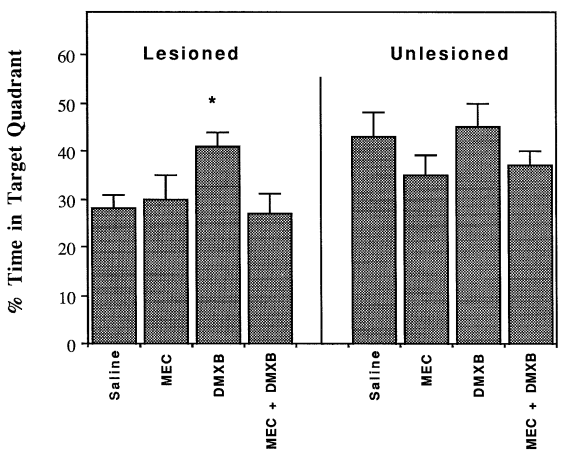

Fig. 5. Effects of DMXB on trial performance in the Morris water task in bilaterally nucleus basalis-lesioned rats. Animals were lesioned, trained and injected as described in Fig. 3. The fraction of time spent in the target quadrant during the 60-s trial interval on day 4 was determined for each group, and expressed as the mean ± S.E.M. of five animals/group. * $P < 0.05$ compared to lesioned group injected with saline (one-way ANOVA).

Table 1
Comparison of Morris water task training latencies among treatment groups with and without nucleus basalis lesions

Treatment	Rank sum statistic vs.:	
	Unlesioned, saline	Lesioned, saline
Unlesioned		
Saline	–	16 [a]
Mecamylamine (0.2 mg/kg)	27	17 [a]
DMXB (0.5 mg/kg)	24	19 [b]
Mecamylamine + DMXB	25	18 [a]
Nucleus basalis-lesioned		
Mecamylamine (0.2 mg/kg)	17 [a]	23
DMXB (0.5 mg/kg)	19 [b]	18 [b]
Mecamylamine + DMXB	20	17 [a]

Animals receiving sham-operations or nucleus basalis lesions were trained in the Morris water task 15 min after specified drug injections (i.p.) as described in the text. The slopes of the resulting acquisition curves from Fig. 3 shown were determined for each animal and given a rank order for comparisons of groups ($n = 5$ animals/group) using the non-parametric rank order statistic.
[a] $P < 0.02$ compared to specified saline control group.
[b] $P < 0.05$ compared to specified saline control group.

manner. Mecamylamine alone had no effect on passive avoidance behavior in lesioned animals (Fig. 3).

DMXB (0.5 mg/kg, i.p.) improved performance in the training (Fig. 4; Table 1) and probe-trial (Fig. 5) phases of the Morris water task in lesioned but not unlesioned animals. DMXB, with or without simultaneous injections of mecamylamine (0.2 mg/kg, i.p.), reduced training-latencies compared to saline-injected, lesioned animals, while mecamylamine alone had no effect (Fig. 4). Rank sum comparisons of these data are described in Table 1. By the end of the 36 trials, all of the groups had similar levels of acquisition (Fig. 4). The improved spatial mem-

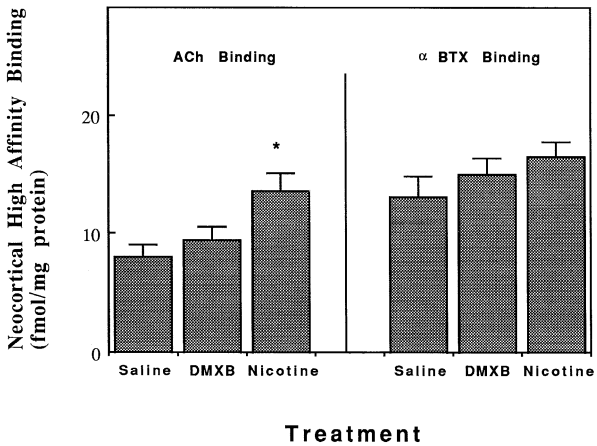

Fig. 6. Effects of chronic injections of DMXB or nicotine on high affinity [^{3}H]ACh or α-[^{125}I]bungarotoxin (BTX) binding in rat neocortices. Adult male rats were injected with saline vehicle, 1 mg/kg DMXB or 0.2 mg/kg nicotine (NIC) (i.p.) daily for 2 weeks. At that time, neocortices were removed and assayed for high affinity [^{3}H]ACh or α-[^{125}I]bungarotoxin binding as described in the text. Each value is the mean ± S.E.M. of four animals/group; * $P < 0.05$ compared to saline-injected control value (one-way ANOVA).

ory observed with DMXB during the probe-trial was blocked by mecamylamine (Fig. 4). No difference in swim distance was observed among the lesioned or unlesioned groups during the testing interval (one way ANOVA values for drug-effect in lesioned group: $F = 0.78$; unlesioned group: $F = 0.42$).

Rat brain high-affinity [^{3}H]ACh binding was elevated after 14 days of daily i.p. injections with 0.2 mg/kg nicotine (1.2 μmol/kg) but not with 1 mg/kg (2.6 μmol/kg) DMXB when compared to saline-injected controls (Fig. 6). Neither drug treatment affected α-bungarotoxin binding density, however. Rat neocortices were harvested 24 h after the last injection to minimize the potential effects of residual drugs or their metabolites on binding density.

4. Discussion

Ibotenate-treated, nucleus basalis-lesioned rats offer a well studied model for memory-related dysfunction. These animals suffer deficits in both avoidance [18,22] and spatial memory tasks [4,8]. However, the neurochemical substrates underlying these two types of behaviors appear to differ. While the loss of cholinergic neurons in the nucleus basalis appears to underlie avoidance behavior deficits, local damage to pallidal or other neurons may account for spatial water task deficits, based on lesion-studies with AMPA and quisqualate [4,22]. Present results indicate that $\alpha 7$ receptor activation is sufficient to improve both types of behavior, consistent with these receptors being localized in several brain regions important for learning and memory, including hippocampus and neocortex.

The present results demonstrate that DMXB activates $\alpha 7$ but not other types of nicotinic receptors in oocyte expression systems, consistent with earlier reports that it displaced high-affinity α-bungarotoxin binding specific for $\alpha 7$ receptors in neocortical tissue and that it activated $\alpha 7$ receptors without activating $\alpha 4\beta 2$ subtypes. DMXB was found previously to overcome nucleus basalis lesions induced deficits in passive and active avoidance behaviors [18]. The present study extends these results to show that the $\alpha 7$-selective DMXB improvement in passive avoidance behavior is mecamylamine-sensitive and therefore requires nicotinic transmission.

These results also demonstrate that this agonist can improve performance in the Morris water task, both in the training and probe-trial phases. Only the probe-trial phase of this paradigm clearly involves spatial learning, since training can involve specially adapted behaviors relevant to this task (e.g., swimming at a constant distance from the wall), as noted previously [19,25]. The ability of mecamylamine to block the DMXB-induced improvement in probe-trial performance indicates that at least one component of this memory-related task (acquisition, retention, or retrieval) is nicotinically mediated. Since mecamylamine was co-administered with DMXB throughout the study, it is impossible to ascertain which of these three memory-related processes is involved.

Mecamylamine had no effect on DMXB-induced training, suggesting that this component of the Morris water task, which involved acquisition and short-term retention of both spatial and non-spatial types of learning, may be mediated by a non-nicotinic action of DMXB or its metabolites in brain. Alternatively, mecamylamine may not have blocked all of the $\alpha 7$ receptors in brain at the dose used. Consistent with this hypothesis was the lack of attenuation of this behavior by mecamylamine in intact animals at the dose used, and the relative low potency of mecamylamine at this receptor subtype [5]. While our results indicate that mecamylamine also has relative low potency at rat $\alpha 7$ receptors compared to other subtypes (where K_i values are typically in the 100 nM range), our results also indicate that antagonist-potency may be underestimated by not pre-applying mecamylamine (e.g., [5]).

The precise fraction of $\alpha 7$ receptors blocked in vivo is difficult to determine in the present study, since the concentration of the drug at synaptic and extra-synaptic sites is unknown. It is nonetheless possible to estimate the peak concentration of the drug in brain-water assuming: (a) equal drug distribution throughout body-water; (b) 70% of body weight consisting of water; and (c) rapid accumulation of drug in brain compared to elimination, then a dose of 0.2 mg/kg (1.2 μmol/0.7 l) would translate to a concentration of about 1.5–2 μM. At this concentration, our oocyte data would suggest about 50% blockade of receptors, which may be enough to attenuate some behavioral actions of these receptors but not others. This dose was selected, it should be noted, because higher concentrations of mecamylamine may block other cationic channels. It is also possible that another mecamylamine-insensitive type of nicotinic transmission may be involved, though this transmission has not been demonstrated.

A cinnamylidene-derivative of anabaseine, 3-[4-dimethylaminocinnamylidene]anabaseine (DMAC) was found recently to activate $\alpha 7$ receptors selectively with greater efficacy than had been reported for DMXB; however, it also blocked these receptors in a use-dependent manner at behaviorally relevant concentrations (e.g., 20 μM) at which DMXB did not [7]. Unlike DMXB, DMAC improved performance in passive avoidance behavior but not active avoidance behavior [18]. Whether this behavioral difference between DMXB and DMAC is related to the mixed agonist/antagonist activity of the latter compound remains to be ascertained. However, it seems clear that the nature of the substituent at the 3 position of anabaseine is important for determining both the behavioral as well as receptor-activation properties of this class of compound.

$\alpha 4\beta 2$ receptors are, next to $\alpha 7$ receptors, the predominant nicotinic subtype in brain based on ligand binding and have been associated with learning and memory related behaviors with selective agonists such as ABT418 [2,6]. These receptors typically increase in density or concentra-

tion following chronic agonist administration [11], which provides an indirect estimate of the putative agonist activity of a nicotinic ligand. They are typically assayed with ligands such as ACh, cytisine, or methacarbachol, under conditions designed to reduce muscarinic or other types of binding [15,21]. Under these conditions, about 90% of the high-affinity binding is associated with $\alpha 4\beta 2$ subtypes [11]. This subpopulation of receptors was elevated in concentration by daily nicotine as expected, but not by DMXB administration, arguing against an activation or desensitization of these receptors by DMXB or any of its metabolites. In addition, neither non-selective nor selective $\alpha 7$ receptor activation appeared sufficient to increase the number of $\alpha 7$ receptors labelled with α-bungarotoxin, a selective ligand for this receptor subtype in neocortex. Since the number of animals per group was not large ($n = 4$) in this study, however, it is possible that more subtle changes occur, e.g., with respect to different layers of neocortex or subpopulations of neurons. These possibilities may be more directly addressed with autoradiographic procedures.

DMXB also had no effect on neocortical high-affinity α-[^{125}I]bungarotoxin binding after 14 days of injections, suggesting this selective activation of $\alpha 7$ receptors was insufficient to increase their binding density. Previous studies have not consistently observed up-regulation of these binding sites in different brain regions, however, unlike high-affinity ACh/nicotine binding sites. Therefore, it may not be surprising that selective $\alpha 7$ activation has little effect on this receptor density.

These results indicate that DMXB may represent a new class of compound with agonist-selectivity for $\alpha 7$ receptors and memory-enhancing properties, both in spatial and avoidance behaviors. It also demonstrates that this receptor-subpopulation may account for at least some of the behavioral properties of nicotine itself relative to memory-related behaviors. Combined with the reported neuroprotective action of DMXB in apoptotic models of trophic factor withdrawal [16], this study suggests that this class of agent will be a useful target for drug discovery relative to the treatment of Alzheimer's disease.

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