Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 369–373, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00406-4

Effects of Nicotinic Dimethylaminoethyl Esters on Working Memory Performance of Rats in the Radial-Arm Maze

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Received 18 January 1994

LEVIN, E. D., J. E. ROSE AND L. ABOOD. *Effects of nicotinic dimethylaminoethyl esters on working memory performance of rats in the radial-arm maze.* PHARMACOL BIOCHEM BEHAV 51(2/3) 369–373, 1995.—Nicotine has been found to improve memory performance in a variety of tests, including the radial-arm maze. This improvement, together with the consistent finding of a decline in cortical nicotinic receptor concentration in Alzheimer's patients, has fueled the search for novel nicotinic ligands with therapeutic potential. In the current studies, a series of nicotinic compounds was tested for effects on working memory performance in the radial-arm maze. One of the three compounds tested, DMAE II (dimethylaminoethanol cyclohexyl carboxylate fumurate), produced significant improvements in working memory performance. In the first experiment, this drug produced a biphasic dose-response curve with improved performance at the 20-mg/kg dose but not at 10 or 40 mg/kg. In a second round of DMAE II administration, the same rats showed a significant improvement with the 40-mg/kg dose. In the second experiment, a new set of rats also showed a biphasic dose-response to DMAE II. The 20-mg/kg dose caused a significant improvement whereas the 40-mg/kg dose did not. Interactions of DMAE II with nicotine and mecamylamine were also studied. Nicotine (0.2 mg/kg) by itself caused a significant improvement in working memory performance. No additive effects of DMAE II with nicotine were seen. In fact, some attenuation of response was seen with the combination. Choice accuracy data for mecamylamine could not be analyzed because of excessive sedation and nonresponding. These studies show that, like nicotine, the nicotinic ligand DMAE II causes an improvement in radial-arm mace choice accuracy. The lack of additivity with nicotine may have been due to the partial agonist effects of DMAE II.

Nicotinic Cholinergic Radial-arm maze

NICOTINE has been found in a wide variety of studies to improve attentional and memory performance [for review see (16)]. However, these effects may be task dependent in that not all studies have detected beneficial effects and some have detected nicotine-induced impairments (10,28,33,35,37). We have conducted a series of studies in the radial-arm maze that examined the effects of nicotine administration on spatial working memory in rats. Acute systemic administration of nicotine (0.2 mg/kg) improved working memory performance of rats in the radial-arm maze (25). We have seen a similar facilitation of choice accuracy in the radial-arm maze after acute intraventricular infusion of nicotine (6). Many other studies have found nicotine-induced improvement in attentional and memory performance in humans (3,31,36,38), monkeys (7,11,13,14), and rats (5,8,12). Nicotine has also been found to improve working memory performance when given on a chronic basis (17,18,20,21,24,29).

Other nicotinic agonists may have similar effects. The nicotinic agonist lobeline has recently been found to improve memory in a passive avoidance task and to effectively attenuate the water maze acquisition deficit seen after septal lesions in rats (9). Recently, a number of carbamate, cycloalkyl, and aryl esters of dimethylaminoethanol, choline, and other aminoalcohols were synthesized that possessed both nicotinic

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cholinergic agonist and antagonist properties as determined by various pharmacological tests in rodents (1,2). The current studies were conducted to assess the efficacy of three dimethylaminoethanol (DMAE) compounds for improving working memory performance in the radial-arm maze.

Novel nicotinic ligands may have similar beneficial effects as nicotine in improving memory function without adverse side effects such as proconvulsant actions, cardiovascular effects, and abuse liability. In addition, examination of a variety of nicotinic ligands that have a range of potencies for improving memory performance will help further the understanding of the nature of the involvement of nicotinic systems in memory function.

METHOD

Subjects

Young adult female Sprague–Dawley strain rats (Zivic-Miller, Allison Park, PA) were used in the present experiment. They were housed in groups of two to four in plastic cages with wood shavings. They had ad lib access to water. They were fed daily after testing such that their weights were kept at 80–85% of free-feeding levels.

Radial-Arm Maze Training

Behavioral testing was conducted on a radial eight-arm maze constructed of wood and painted black. The central arena was 50 cm in diameter and eight 10 × 60-cm arms extended radially; food cups were located 2 cm from the distal end of each arm. The maze was positioned 30 cm above the floor in a testing room that contained many extra-maze visual cues.

The rats were tested 3–5 days per week. Before the session, each arm of the maze was baited with a one-third to one-half piece of sugar-coated cereal (Kellogg's Froot Loops®). At the beginning of the session, the rat was placed in a circular plastic ring in the central platform; after 10 s, the ring was lifted and the rat was allowed to freely walk through the maze. Arm choices were recorded when the rat had placed all of its paws beyond the threshold at the proximal end of the arm. Because the reinforcements were not replaced during the session, only the first entry in each arm was rewarded. Subsequent reentries were scored as errors. The session continued until the rat had entered all eight arms or 5 min had elapsed. The choice accuracy measure was the number of entries until an error was made (entries to repeat). The response duration measure was the total session duration divided by the number of arms entered (seconds per entry).

Drug Treatment

After the 20 sessions of acquisition, the rats began the drug studies with the DMAE compounds. The synthesis, receptor binding properties, and pharmacology of the dimethylaminoethyl benzoate fumurate (DMAE I), dimethylaminoethyl cyclohexylcarboxylate bifumurate (DMAE II), and dimethylaminoethyl phenylcarbamate bifumurate (DMAE III) have been described elsewhere (2). The chemical structures are shown in Fig. 1. The drugs were dissolved in 0.9% sterile saline, which was administered by itself for control injections. Doses of all the drugs are expressed as a function of the salt weight. Experiment 1a used a repeated-measures counterbalanced design in which the rats (N = 11) were acutely administered (SC) 0, 10, 20, and 40 mg/kg of DMAE I, II, and III with the doses calculated on the weight of the bifumarate salt. The

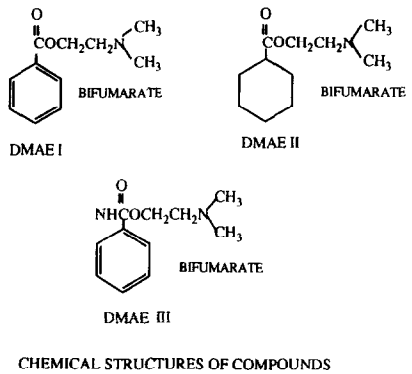

FIG. 1. Chemical structures of DMAE I, DMAE II, and DMAE III.

molar equivalents of these doses are 22.0, 44.0, and 87.9 μmol/kg for DMAE I; 21.6, 43.3, and 86.6 μmol/kg for DMAE II; and 21.3, 42.6, and 85.1 μmol/kg for DMAE III. In Experiment 1b the same 11 rats were tested again with the 0-, 10-, 20-, and 40-mg/kg doses of DMAE II in a counterbalanced order. Experiment 2 used a repeated-measures counterbalanced design in which a different set of rats (N = 9) was acutely administered (SC) 0, 20, and 40 mg/kg of DMAE II alone or together with 0.2 mg/kg of nicotine ditartrate or 10 mg/kg of mecamylamine hydrochloride. Two subjects that did not make sufficient arm entries to calculate choice accuracy data with the 20-mg/kg DMAE II dose were removed from data analysis. Experiment 3 (N = 10) was conducted to add power to the overall analysis of the effect of the 20-mg/kg dose of DMAE II on choice accuracy in the radial-arm maze and to determine the differential effect of DMAE II on rats performing at good and poor levels of accuracy. In all three experiments, injections were given in a counterbalanced order 20 min before testing in a volume of 1 ml/kg. There were at least 2 days between drug injections.

Statistics

The choice accuracy and response duration measures were assessed by a within-subjects design analysis of variance (ANOVA). Planned comparisons were made between the drug combinations and individual drug doses used (15). A value of $p < 0.05$ (two-tailed) was considered significant. A value between $p = 0.10$ and $p = 0.05$ (two-tailed) was considered to be marginally significant and suggestive of an effect.

RESULTS

Experiment 1a

As shown in Fig. 2, DMAE II showed a biphasic dose effect where the 20-mg/kg dose caused a marginally significant, $F(1, 30) = 3.69$, $p < 0.07$, improvement relative to saline and the 40-mg/kg dose did not cause any improvement. No effects were detected with DMAE I and DMAE III. No significant effects were seen in terms of response latency.

Experiment 1b

Figure 3 shows the effect of DMAE II on choice accuracy when the dose range was given for a second time to the same rats in Experiment 1a. A significant improvement, $F(1, 30) = 5.46$, $p < 0.05$, was induced by the 40-mg/kg dose of DMAE II. This dose was not found to significantly alter latency.

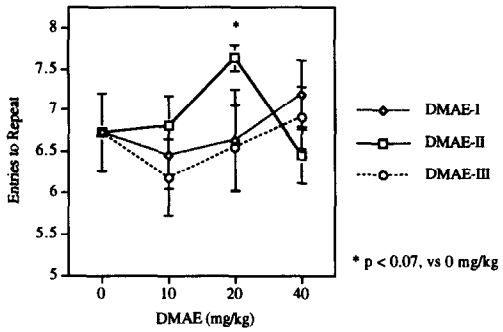

FIG. 2. Experiment 1a. DMAE I, DMAE II, and DMAE III effects on choice accuracy (entries to repeat) ± SEM.

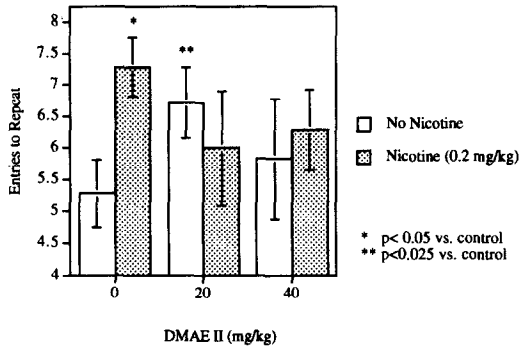

FIG. 4. Experiment 2. Interactions of DMAE II with nicotine and mecamylamine on choice accuracy (entries to repeat) ± SEM.

However, the lower two doses did slightly though significantly ($p < 0.05$) increase response latency: saline = 15.1 ± 0.6 s; DMAE II 10 mg/kg = 18.6 ± 1.6 s; DMAE II 20 mg/kg = 19.2 ± 1.4 s; and DMAE II 40 mg/kg = 15.9 ± 0.9 s.

Experiment 2

As in Experiment 1a, 20 mg/kg of DMAE II produced a significant, $F(1, 6) = 8.82$, $p < 0.025$, improvement in choice accuracy (Fig. 4). Also as in Experiment 1a, the high dose of 40 mg/kg of DMAE II did not produce an improvement. Replicating earlier studies, nicotine (0.2 mg/kg) by itself significantly, $F(1, 6) = 6.46$, $p < 0.05$, improved choice accuracy. The 20-mg/kg dose of DMAE II did not add to the effect of nicotine. In fact, both the 20- and 40-mg/kg doses of DMAE II attenuated the effect of nicotine such that it was not different from control. However, no significant deficits relative to nicotine alone were seen with the addition of DMAE II. Mecamylamine caused excessive sedation and balking such that choice analyses could not be conducted. There were no significant effects of nicotine or DMAE II on response latency.

Experiment 3

There was a similar trend of improvement with the 20-mg/kg dose of DMAE II. With saline the rats averaged 5.67 ± 0.56 entries to repeat whereas with DMAE II they averaged 6.22 ± 0.42 entries to repeat. The overall main effect of DMAE II did not reach significance in this experiment, but there was a significant DMAE × performance level interaction, $F(1, 8) = 8.35$, $p < 0.025$. The rats with poorer accuracy at the end of the predrug acquisition training showed significant improvement with DMAE II (saline = 4.40 ± 0.68, DMAE = 6.44 ± 0.23), whereas the rats with better accuracy at the end of the predrug acquisition training were not significantly affected by 20 mg/kg of DMAE II (saline = 6.93 ± 0.37, DMAE = 6.00 ± 0.84). There was a significant negative correlation of the level of predrug acquisition accuracy with improvement caused by 20 mg/kg of DMAE II ($r = 0.792$, $p < 0.01$). As in the previous experiments, no effect of DMAE II was seen in terms of latency.

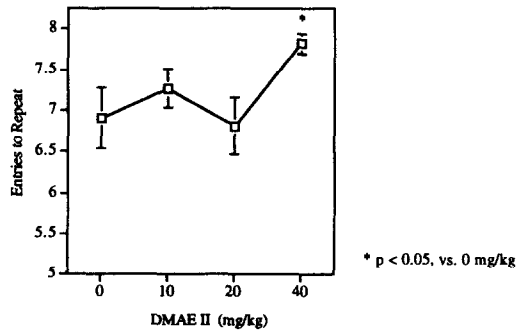

FIG. 3. Experiment 1b. DMAE II effects on choice accuracy (entries to repeat) ± SEM.

To determine the overall effect of the 20-mg/kg dose of DMAE II on choice accuracy, the data from all three experiments for saline and this dose were analyzed together. For Experiment 1, where rats were given saline and 20 mg/kg of DMAE II twice (Exp. 1a and Exp. 1b), the scores were averaged for analysis. This overall analysis of 28 rats demonstrated a significant improvement caused by 20 mg/kg of DMAE II, $F(1, 27) = 5.67$, $p < 0.025$. With saline the rats averaged 6.02 ± 0.29 entries to repeat and with 20 mg/kg of DMAE II the rats averaged 6.74 ± 0.22 entries to repeat.

DISCUSSION

The DMAE II-induced facilitation of radial-arm maze choice accuracy seen in the present study is similar to previous findings that nicotine can improve memory performance [for review see (16)]. We have previously found that both acute (6,25) and chronic (17,18,20,21,24) nicotine administration improves working memory function as measured by the radial-arm maze. Unlike 20 mg/kg, the higher DMAE II dose of 40 mg/kg did not cause a significant improvement in Experiment 1a. An inverted U-shaped dose-response curve is typical for drugs that improve cognitive function. In particular, drugs that stimulate the nicotinic receptor at low doses may inhibit it at higher doses because this receptor is easily desensitized (30). Interestingly, in Experiment 1b, when the rats were given the drugs a second time, the dose-response curve was shifted to the right. The high dose of 40 mg/kg of DMAE II significantly improved performance whereas the lower dose of 20 mg/kg was ineffective. Mechanisms underlying this apparent tolerance are currently obscure.

The inverted U-shaped curve with DMAE II may also be due to the fact that the agent appears to be a mixed nicotinic agonist-antagonist. Behavioral studies in rats reveal that the agent, when administered intraventricularly, is able to both produce prostration characteristic of nicotine and to partially

TABLE 1
DMAE COMPOUNDS ACh RECEPTOR BINDING

	(^{3}H)Nicotine	(^{3}H)QNB
DMAE I	1×10^{-6}	2×10^{-5}
DMAE II	2×10^{-6}	2×10^{-6}
DMAE III	1×10^{-4}	5×10^{-6}

prevent nicotine-induced prostration. When administered intraperitoneally, it is also able to partially prevent nicotine-induced seizures and mortality (2). DMAE II may have acted as an agonist prior to the nicotine administration desensitizing the nicotinic receptor and thus attenuating subsequent nicotine effects. However, arguing against this explanation, the current results show that DMAE II attenuates nicotine effects on memory performance when given simultaneously. The failure of DMAE III to improve working memory may be due to the fact that its affinity for brain nicotinic receptors is 1/50 that for DMAE II (2). Although the affinity of DMAE I for nicotinic receptors is comparable to DMAE II, the ineffectiveness of DMAE I in improving memory performance may be due to the fact that DMAE I is a more potent nicotinic antagonist than DMAE II. There may also be effects of these compounds mediated via muscarinic as well as nicotinic receptors. The K_is for the three DMAE compounds for ACh receptors have been previously determined (2,4) and are shown in Table 1. The effects of these drugs at muscarinic sites may also be involved in the observed cognitive effects. The specific mechanisms are still under investigation.

In Experiment 2 the memory-improving effect of DMAE II was replicated in another set of rats. The biphasic effect of DMAE II on choice accuracy with the 20-mg/kg dose improving performance was very similar to what was seen in Experiment 1a. Also replicating our earlier work (25), an acute dose of 0.2 mg/kg of nicotine was found to improve working memory performance in the radial-arm maze. It is interesting that the DMAE II and nicotine did not cause a mutually potentiating facilitation of choice accuracy. This may reflect the inverted U-shaped curve seen with increasing doses of DMAE and nicotine. Perhaps lower dose combinations of DMAE II and nicotine would have additive effects. Alternatively, perhaps nonnicotinic mechanisms are critical for the DMAE effect. There was greater trouble with the sedative effects of mecamylamine in Experiment 2 than with previous ones (19,22–24,26,27). Perhaps giving it in conjunction with DMAE II, which, as mentioned, seems to have partial antagonist properties, accounted for this.

Experiment 3 further characterized the positive effect of the 20-mg/kg dose of DMAE II. The rats that performed less well on the predrug acquisition training were selectively improved by the drug. The overall analysis showed a clear improvement of working memory performance caused by DMAE II. This compound may be useful for addressing the cognitive deficits associated with nicotinic receptor loss such as Alzheimer's disease (32,34,39).

ACKNOWLEDGEMENTS

The authors thank Channelle Christopher and Sandra Briggs for their help in testing the animals.

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