Effect of Dextromethorphan on Reference Memory Assessed in Rats by a Three-panel Runway Task

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Summary

The effects of dextromethorphan (DM, CAS 6700-34-1), a common over-the-counter cough suppressant, on the reference memory have been investigated by a repeated acquisition procedure such as a radial-arm maze task or a water maze task. DM (20–40 mg/kg i.p.) produced a significant decrease in the number of errors (pushes made on the two incorrect panels of the three panel gates at four choice points) and latency. Systemically administered scopolamine (CAS 114-49-8) (1 mg/kg i.p.) impaired the performance on both parameters. DM (40 mg/kg i.p.) was effective in reversing the reference memory deficit induced by administration of scopolamine. DM acts as a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptors. Our results suggest that inhibition of NMDA receptors by DM supports its potential positive properties. This finding might present an opportunity for the evaluation of this old antitussive drug.

Key words

- Antitussive, non-opioid
- CAS 114-49-8
- CAS 6700-34-1
- Dextromethorphan, effect on memory, rat, three-panel runway task
- Scopolamine

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1. Introduction

Dextromethorphan (DM, CAS 6700-34-1) is a non-opioid antitussive drug safely used in the clinic for more than 25 years [1]. In addition, it has been shown to possess anticonvulsant and neuroprotective effects in a variety of experimental models [2−5]. It has been shown that DM protects against hypoxia-ischemia cerebral infarction in a rat model [6] and in humans [7]. Although much research has been directed to the treatment of dementia and cognitive dysfunction, the underlying mechanisms of the effects of some drugs such as memantine are not clearly understood yet. Memantine and the common antitussive drug DM can act as non-competitive N-methyl-D-aspartate (NMDA) antagonists [8, 9].

Long-term potentiation (LTP) in the hippocampus is an appropriate model of synaptic plasticity and is widely believed to correlate with learning and memory. Activation of NMDA receptors is believed to be required for the induction of LTP, which may underlie the storage of information in the brain and may be critical for learning and memory [10]. Both the competitive and non-competitive NMDA receptor antagonists have been shown to impair learning and memory formation in various behavioral tasks in experimental animals. The effects of intrahippocampal injections of the selective and competitive NMDA receptor antagonists CGS 19755 (cis-4-phosphonomethyl-2-piperidine carboxylic acid), CPP and AP5 on working and reference memory, have been investigated in rats by the three-panel runway task [11]. These findings indicate that mechanisms mediated by NMDA receptors in the hippocampus are involved in working memory, but not in reference memory [11]. It has been shown that MK-801, another non-competitive NMDA antagonist, produces an anterograde amnesia in the elevated-plus maze test in mice [12]. In another study, potent competitive NMDA receptor antagonist CGP 40116-induced memory impairments of mice have been shown in water maze and passive avoidance task [13]. Parada-Turska and Turski [10] examined competitive NMDA antagonists, CGS 19755 and CPP in memory processes. Their findings suggested that the NMDA antagonists CGS 19755 and CPP may impair learning (storage) but have little or no effect on recall (retrieval) from long-term memory, in Y-shaped maze and a step-through passive avoidance task in mice.

It has been proposed that there are two different aspects of memory in experimental animals, i.e. working memory and reference memory. Working memory allows animals to remember information that is useful for a single session of an experiment but not for subsequent sessions, whereas reference memory is defined as the holding information that is of continued value across all sessions [14, 15]. Furuya et al. 1988 [16], Yamamoto et al. 1990 [17], Ohno et al. 1991 [11] and Nakazato et al. 2000 [18] suggested that the three-panel runway task serves well as a method for the study of learning and memory functions in rats.

2. Materials and methods

2.1. Animals

Wistar Albino male rats were obtained from the Experimental Medical Research Unit (DETAB, Kocaeli University, Kocaeli, Turkey). Adult rats were used weighing between 210 and 250 g at the start of food deprivation. Then they were placed on a deprivation schedule to maintain their weights at approximately 80% of the free-feeding level. Rats were housed with free access to water in groups of 4 per cage in a 12-h light/dark cycle (08:00–20:00 h light) at a constant temperature (21 ± 4 °C).

2.2. Drugs

Dextromethorphan hydrobromide and scopolamine hydrobromide (Sigma Chemical, St. Louis, MO, USA) were dissolved in saline. Drugs and saline were injected intraperitoneally (i.p.) at a volume of 0.5 ml/200 g body weight. The doses chosen were adapted from other studies that have examined the effect of DM and scopolamine [16, 19, 20]. The runway test was given 30 min after i.p. injections of scopolamine and 60 min after i.p. injections of DM.

2.3. Apparatus

Reference memory was assessed with a three-panel runway apparatus which was introduced previously [16, 17, 18, 21]. In brief, this apparatus (175 × 36 × 25 cm) was composed of a start box, a goal box and four consecutive choice points intervening between them. Each choice point consisted of a gate with three panels (12 × 25 cm). The rats were put into the start box and then they were allowed to run the task after the guillotine door was opened in front of the start box. The rats were prevented from passing through two of the three panels in the gate by front stoppers and prevented from returning to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all the gates. When the rats reached the goal box, they received two food pellets (about 50 mg each) as positive reinforcement [11].

2.4. Acquisition training

Initially, all the front stoppers were removed so that a rat could pass through any one of the three panel gates at each choice point. The rats were made to run the task repeatedly until the time that elapsed from leaving the start box to reaching the goal box was consistently below 20 s. Once this time was reached, the rats were given six consecutive trials (one session) per day with the removal of the front stopper of only one of the three panel gates (the correct panel gate). Trials were run at 2-min intervals, during which time the animals were returned into the home cage. In the reference memory procedure, the correct panel gate locations were kept constant both within and across sessions. The number of times an animal pushed an incorrect panel gate (errors) and the time required for the animal to obtain food pellets (latency) were recorded for each rat in every trial of a session. The criterion of learning was less than 6 errors summed across the six trials of a session. After the rats achieved this criterion throughout three consecutive sessions, they were used in the experiment (n = 7 or 8 for each group). All experiments were carried out at the same time every day during the light period (9:00–12:00). The ethical approval was granted by the Kocaeli University Ethics Committee (Kocaeli, Turkey).
2.5. Data analysis
The number of errors and the latency were summed across all six trials of a session. The presence of a significant difference between the groups was determined by a one-way analysis of variance (ANOVA) that was followed by Tukey test when F ratios reached significance (p < 0.05).

3. Results
3.1. Acquisition processes
The number of errors was gradually decreased from trial 1 to 6. The number of errors and latency in all six trials of a session also decreased with the repetition of training (Fig. 1 and 2). The rats could run the task within the six-error criterion summed across six trials after they had 10–15 training sessions.

3.2. Effects of systemic DM and scopolamine administration
Scopolamine (1 mg/kg i.p.) significantly increased the number of errors [F(2,19) = 67.957, p < 0.01] and latency [F(2,19) = 25.940, p < 0.01] in the three-panel runway performance (Table 1). Total errors summed across six trials of a session in scopolamine-injected rats were 19.9 ± 2.0 (mean ± SEM). DM (10 mg/kg i.p.) did not change the number of errors and latency from saline level (Fig 3, 4; Table 1). DM (in doses of 20 and 40 mg/kg i.p.) caused a significant reduction of the number of errors [F(3,24) = 10.130, p < 0.05] and latencies [F(3,24) = 6.124, p < 0.05] in the three-panel runway performance (Fig 3, 4; Table 1). In addition, DM (40 mg/kg i.p.) reversed the effect of scopolamine (1 mg/kg) on the increases in errors and latency (Fig 5, 6; Table 1). Total errors summed across six trials of a session in rats injected with scopolamine after DM were 2.0 ± 0.7 (mean ± SEM, p < 0.01). We previously reported that DM (40 mg/kg i.p.) did not produce any significant change in locomotor activity in the naive rats [19].

4. Discussion
This study has demonstrated that DM can reverse scopolamine-induced memory deficit on a three-panel runway task in rats. Wang et al. had previously reported that dlimemorfan, an analogue of DM, could attenuate scopolamine amnesia in a step-through passive avoidance test as well as in a water maze test in mice [1]. Although different explanations may be related to different administration schedules, amnesia models, and behavioural test, it is well known that the glutamatergic system plays an important role in the learning and memory processes.

L-Glutamate is the main excitatory neurotransmitter in the central nervous system, implicated in learning, memory processes and neuronal plasticity [22]. It is suggested that enhancement of stimulation of NMDA receptors plays a role in the pathogenesis of Alzheimer’s disease. Low affinity NMDA type receptor antagonists, such as memantine, might prevent excitatory amino acid neurotoxicity without interfering with the physio-

Table 1: Effect of i.p. injections of DM and scopolamine (SC) on errors and latency assessed by a reference memory procedure in the three-panel runway task.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg i.p.)</th>
<th>n</th>
<th>No. of errors Trial 1–6</th>
<th>Latency (s) Trial 1–6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>7</td>
<td>2.9 ± 0.3</td>
<td>44.6 ± 2.9</td>
</tr>
<tr>
<td>DM10</td>
<td>10</td>
<td>7</td>
<td>2.7 ± 0.5</td>
<td>40.3 ± 4.8</td>
</tr>
<tr>
<td>DM20</td>
<td>20</td>
<td>7</td>
<td>1.4 ± 0.3*</td>
<td>28.1 ± 1.8**</td>
</tr>
<tr>
<td>DM40</td>
<td>40</td>
<td>7</td>
<td>0.6 ± 0.3*</td>
<td>26.1 ± 4.3**</td>
</tr>
<tr>
<td>DM40+SC</td>
<td>40 + 1</td>
<td>8</td>
<td>2.0 ± 0.7</td>
<td>72.3 ± 25.1**</td>
</tr>
<tr>
<td>SC</td>
<td>1</td>
<td>7</td>
<td>19.9 ± 2.0**</td>
<td>263.0 ± 35.5**</td>
</tr>
</tbody>
</table>

The runway test was performed after the drugs had been administered. Each value represents the mean ± S.E. of errors and latencies summed across all six trials of a session. The significance of the differences from saline-treated group was determined by a one-way analysis of variance followed by Tukey test. * p < 0.05, ** p < 0.01.
logical action of glutamate that are required for learning and memory function [23]. Memantine was an approved drug for the treatment of moderately severe to severe Alzheimer’s disease.

Generally, NMDA receptor antagonists are not suitable for parenteral administration because they do not easily cross the blood-brain barrier. However, DM is a systemically effective non-competitive blocker of NMDA receptors [6]. DM is still used as a nonprescription cough suppressant. Several antipsychotic and antidepressant drugs and certain anticonvulsants also have been shown to interact with the high affinity DM site [24]. DM is virtually devoid of opioid activity [25]. Therefore, DM does not possess the central nervous system effects such as analgesia, respiratory depression, and abuse liability or psychotomimetic properties.

In our previous study we showed that DM attenuates ethanol withdrawal signs in rats [19]. In naive rats, DM did not produce any significant effect on locomotor activity. DM (40 mg/kg) reversed the impairment of learning and memory induced by the blockage of muscarinic cholinergic receptors by scopolamine. Scopolamine, a muscarinic acetylcholine receptor antagonist, is used widely to investigate cholinergic influence on the learning ability in experimental animals. We could not conclude from the present findings how DM acts on cholinergic systems and counteracts the impairment of the reference memory performance in rats. Both acetylcholine and glutamate are thought to play important roles in memory, but the nature of the involvement of cholinergic and glutamatergic systems of the hippocampus in learning and memory processes are still un-
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NMDA receptors have been shown to control the release of dopamine at this synapse. DM protected mesencephalic cell cultures against glutamate [27]. It has a neuroprotective effect on an in vivo model of Parkinson's disease. In another study, systemic administration of (R, S)-1-aminoindan-1,5-dicarboxylic acid (AIDA), a highly selective and potent antagonist of the glutamate receptor, disrupted hippocampus-dependent contextual fear conditioning learning without affecting the hippocampus-independent cued conditioning in rats [28]. Cycloserine is known to enhance NMDA responses through acting as a partial agonist at the glycine modulatory site on the NMDA receptor/channel complex [29]. It was effective in reversing the working memory deficit induced by intrahippocampal administration of scopolamine. These results suggest that the NMDA receptor/channel plays an important role in regulating memory processes. However, Hiramatsu et al. [30] suggested that the reference memory and working memory performance act in different brain areas.

The effects of DM on spatial learning had assessed using the Morris water maze [31]. DM impaired learning of rats dose dependently in the initial training phase of the experiment. Block and Schwarz also showed that in the water maze DM attenuated the deficits in spatial learning and memory following global ischemia [32]. Dematteis et al. reported that DM (10–30 mg/kg) did not impair reference memory in the Morris water maze [33]. It is not clear why these groups have found different results. The differences between these results may be attributed to the mode of drug administration and different assessments of learning and memory procedures.

The human use of DM has been limited to alleviation of the symptoms of common cold. But, DM has been clinically studied as potential treatment for neurodegenerative diseases, chronic pain and epilepsy. The results of our study suggest that DM may be of clinical use in the treatment of Alzheimer's disease in man. Additional human trials to evaluate the use of DM are required.

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Literature


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