EFFECT OF 4-AMINOPYRIDINE DERIVATIVES WITH A PEPTIDE MOIETY ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN MICE

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Abstract. Alzheimer’s disease (AD) is a progressive neurodegenerative disease affecting learning and memory processes. The most commonly used drugs for symptomatic treatment of AD are the inhibitors of the enzyme acetylcholinesterase (AChE) – Galantamine, Rivastigmine, Donepezil. The aim of this study is to investigate the effects on locomotor activity and passive avoidance after single and repeated administration of the compounds in mice. The results demonstrate that newly synthesized compounds that are hybrid compounds including 4-aminopyridine and also modified dipeptides holding the residue of the N-(3, 4-dichlorophenyl)-D,L-Ala-OH, decrease the locomotor activity of mice at doses representing 1/20 of LD50 of the respective substances. The memory improving potential of the compounds was examined using the passive avoidance test and for deterioration of memory processes was used scopolamine in dose 3 mg/kg. The results indicate that some of the compounds antagonize the effects of scopolamine after single and repeated administration.

Keywords: scopolamine, 4-aminopyridine dipeptide derivatives, locomotor activity, passive avoidance

Introduction

Alzheimer’s disease (AD), the most common cause of dementia and neurodegenerative diseases, is characterized by progressive memory impairment [1] and other progressive disturbances of cortical functions including learning capacity, orientation and language [2]. Risk factors associated with AD include age, family history and environmental factors [3].

Besides the neuropathological hallmarks of the disease, neurofibrillary tangles and neuritic plaques, AD is characterized by a consistent deficit in cholinergic neurotransmission particularly in the basal forebrain. The therapeutic strategies to ameliorate AD related symptoms have been aimed to increase acetylcholine (ACh) concentration. Therefore, the cholinergic receptor agonists (muscarinic and nicotinic) and enhancers of endogenous levels of ACh (synthesis promoters and inhibitors of its metabolizing enzyme) have been tested in order to treat senile dementia of Alzheimer type. Among the various approaches attempted to increase cholinergic activity, the inhibition of acetylcholinesterase is the most successful one [4]. The different cholinesterase inhibitors currently available exhibit different pharmacological profiles – tacrine and rivastigmine inhibit acetylcholinesterase and butrylcholinesterase activities, donepezil is highly selective for AChE and galantamine acts as a weak cholinesterase inhibitor [5]. The aim of this paper is to establish the effect of 4-aminopyridine derivatives comprising peptide moiety on scopolamine-induced memory impairment in
mice using locomotor activity and passive avoidance tests.

Materials and methods

Animals

Experiments were performed on male albino mice line H, 27-32 g body weight, supplied by Experimental and breeding base for laboratory animals - Slivnitsa, Bulgaria. Mice were housed by groups in standard polycarbonate cages; water and food were available *ad libitum*, room temperature 22 ± 3°C, humidity 30 %, lighting schedule 12 h light/dark cycle. Animals were trained and tested during light part of the cycle. All experiments were approved by the Institutional Animal Care Committee at the Medical University of Sofia, Bulgaria.

Locomotor activity

Locomotor activity was assessed in plexiglas activity cage – Ugo Basile Biological Research Apparatus, Milan, Italy (38 cm long, 25 cm wide, 23 cm high) that was fitted with grid bars running parallel to each other. When mice cross these bars they activate a circuit which transforms these movements into signals that can be registered as counts per a present time. Groups of 6 male mice each were placed in the activity cage 5 min after the intraperitoneal (i.p.) injection of 1/20 of LD50 of the respective compound. The locomotor activity of scopolamine was measured 5 and 30 minutes after i.p. administration. The mobility of each group was measured for 1 hour and the total number of counts during each 10-min period was recorded. The effect of each compound on locomotor activity was expressed as counts/10 min for 1 hour [7].

Passive avoidance after single administration of the compounds

The scopolamine test was performed in groups of 6 male mice weighing 25-32 g in a one-trial, passive avoidance (PA) paradigm. Five minutes after the i.p. administration of 3 mg/kg scopolamine hydrobromide, each mouse was placed in the bright part of a two-chambered apparatus for training (Gemini Avoidance System, San Diego, California, USA). After a brief orientation period, the mouse usually enters the dark chamber. Once inside the dark chamber, the door is closed which prevents the mouse from escaping, and a 1 mA, 3 seconds foot shock is applied through the grid floor. The mouse is then returned to the home cage. Twenty-four hours later, testing is performed by placing the animal in the bright chamber. The latency time in seconds for entering the second darker chamber within a 5 min test session is measured electronically. The test compounds were administered 90 min before training in a dose 1/20 of LD50. Acquisition trial is the first trial before administration of scopolamine and compounds. On the second day the trial is conducted 5 minutes after scopolamine administration in dose 3 mg/kg i.p. and the retention trial is conducted on the third day 24 h after scopolamine and 90 min after i.p. administration of the investigated compound [8].

Passive avoidance after repeated administration of compounds

The experiments were carried out on 36 male mice weighing 25-32 g divided into 6(n=6) groups. The training degree and dynamics were examined by the method of step-through passive avoidance test with an automated shuttle-box (Gemini Avoidance System, San Diego, CA, USA), divided into a lighted compartment and a dark compartment of the same size (25 cm long x 20 cm wide x 16 cm high) by a wall with a guillotine door (8 cm wide x 6 cm high). Briefly, in the training trial, a mouse was placed in the lighted chamber, and when the mouse entered the dark chamber, a 1 mA electrical shock of 3 s duration was delivered through floor grids. After the training day each animal was trained for five consecutive days (day 1 to day 5) under the same conditions. The tested compounds were administered intraperitoneally 30 min before the passive avoidance test from day 1 to day 5 and after that every day for 7 days without testing the animals in apparatus. Memory impairment was induced by treatment with scopolamine (3 mg/kg, i.p.) 30 minutes after administration of compounds, and
the passive avoidance test was performed 30 min after treatment with scopolamine. Scopolamine was administered on day 1, day 5 and day 12. After five days of training the memory storage processes were studied seven days later again by passive avoidance test in Gemini apparatus [9, 10].

**Drug administration**

The solutions were *ex tempore* prepared with saline and were introduced intraperitoneally in a volume of 0,1 ml/10 g body weight. Scopolamine was administered at dose 3 mg/kg. The newly synthesized compounds, galantamine and 4-AP were administered at dose 1/20 of LD50 of the respective compound. The control group of animals received the dissolver (sodium chloride 0.9%) in the same volume. Drug treatment groups: control group – sodium chloride 0,9%; scopolamine - 3mg/kg; 4-AP – 0,3 mg/kg; galantamine – 0,75 mg/kg; compound 4a - 21,5 mg/kg; compound 4b -15 mg/kg; compound 4c – 4,4 mg/kg; compound 4d – 14 mg/kg.

**Statistical analysis**

The statistical analysis was performed by Student’s (paired) t-test. In the analysis of differences between groups, values of \( p \leq 0,05 \) and \( p \leq 0,01 \) were considered statistically significant.

**Results**

The investigated compounds were synthesized and characterized previously according to Scheme 1 [6]. These compounds comprise 4-AP and also modified dipeptides holding the residue of the N-(3,4-dichlorophenyl)-D,L-Ala-OH.

**Scheme 1.** Synthesis of new dipeptide derivatives of 4-AP.

**Effect on spontaneous locomotor activity.** The locomotor activity in scopolamine treatment groups was determined in control group, 5 and 30 minutes after i.p. administration of scopolamine in dose 3 mg/kg (Figure 1). Stimulation of locomotor activity was recorded between 20th and 40th minute and between 30th and 50th minute in different groups. In these time periods, an almost double increase in motor activity was observed compared to the control group.
Fig. 1. Effect of scopolamine (3 mg/kg) on locomotor activity 5 and 30 minutes after i.p. administration. The effect of each compound on locomotor activity was expressed as counts/10 min for 1 hour.

The locomotor activity of newly synthesized compounds was recorded 5 min after i.p. administration of 1/20 part of LD50. All compounds caused reduction in spontaneous locomotor activity in mice within 1 hour test period, as compared with the activity of control mice receiving saline only (Figure 2).

Fig. 2. Effect of 4-AP derivatives on locomotor activity after i.p. administration of 1/20 of LD50. The effect of each compound on locomotor activity was expressed as counts/10 min for 1 hour.
Fig. 3. Effect of a single administration of newly synthesized compounds, galantamine and 4-AP on scopolamine-induced memory deficit in the passive avoidance task. Acquisition trial is the first trial before administration of scopolamine and compounds. On the second day the trial is conducted 5 minutes after scopolamine administration in dose 3 mg/kg i.p. and the retention trial is performed on the third day 24 h after scopolamine and 90 min after i.p. administration of the investigated compound. The maximal time of latency was 300 s. Values are expressed as mean ± SEM; n = 6.

* p≤ 0.05 statistically significant difference in latency time on acquisition trial as compared to the trial conducted 5 min after scopolamine administration; Student’s (paired) t-test.

**p≤ 0.01 statistically significant difference in latency time on acquisition trial as compared to the trial conducted 5 min after scopolamine administration; Student’s (paired) t-test.

It was observed that scopolamine administered i.p. at dose 3 mg/kg 5 minutes before the test in scopolamine group prolonged the latency of entering the dark chamber. This prolongation is almost two times more pronounced (p≤ 0.01) compared to the one in the acquisition trial. Data from the 3rd day showed impaired retention test performance 24 h after scopolamine administration. The compounds 4c (p≤ 0.05) and 4a increase the latency of entry in the dark chamber. The most pronounced effect is observed in the following sequence: 4c > 4a > Galantamine. The other three compounds did not show any significant change (Figure 3).
The results indicated decrease in latency times in learning sessions between 1st and 5th day after drug administrations except by the compound 4d. The increase in latency times was observed in all groups except 4a and 4d groups on the 12th day, when memory retention test was performed. 4b and 4c counteracted the effect of scopolamine especially on the 12th day, when is the retention of memory (Figure 4).

Discussion

The results show that the 4-AP derivatives decrease the locomotor activity after the 20th minute of 1 hour test session. An increase in locomotor activity was observed in scopolamine group which is in line with the data of Katz et al., Itzhak and Martin, and Shimosato et al.[11, 12 13], who reported that the non subtype-selective muscarinic antagonist scopolamine and the M1/M4-preferring antagonist trihexyphenidyl both induced hyperlocomotion, and both increased cocaine- or methamphetamine-induced hyperlocomotion in mice.

Lynch et al. reported the effects of chlorpromazine and caffeine on spontaneous locomotor activity in rats obtained from two automated test systems. Chlorpromazine decreased spontaneous locomotor activity in a dose-dependent manner, with statistically significant effects on horizontal activity at doses 8 and 16 mg/kg b.w. after single administration. By comparison, caffeine produced dose-dependent increases in spontaneous locomotor activity, with statistically significant effects on horizontal activity at doses 6, 12 and 24 mg/kg b.w. [14].

Passive avoidance

A prolonged latency in passive avoidance indicates that the animal remembers that it has been punished and, therefore, does avoid the darker chamber and on the other hand a shortened latency indicates worsening in the learning of the task. Antimuscarinic drugs have been extensively used as amnesic drugs in animals to mimic the cognitive dysfunction observed in dementia and AD. The majority of the studies focused on scopolamine-reversal as initial screening method to identify therapeutic candidates for cognitive disorders. Scopolamine is a tropane alkaloid drug with competitive antagonism at the muscarinic acetylcholine receptors (mAChR)[15]. Muscarinic antagonists disrupt performance on several reference memory tasks, such as active and passive avoidance,
object discrimination, radial arm maze, water maze (WM) and fear conditioning [8, 16, 17, 18].

The results from the passive avoidance test after single administration of the test compounds showed that scopolamine administered subcutaneously at dose 3 mg/kg 5 minutes before the test in scopolamine group, prolonged the latency of entering the dark chamber. This prolongation is almost two times more pronounced than this in the acquisition trial. Data from the 3rd day showed impaired retention test performance 24 h after scopolamine administration. Possible explanation of these results we could find in different studies. The research literature indicated that scopolamine can either impair or improve learning and memory and by using the same strain of mice, task, and training procedures, scopolamine improved or impaired retention test performance depending on the dosage and time of administration [19]. Elrod et al. reported that rats treated 30 minutes before training trial with 0.4 mg/kg and 1.2 mg/kg resulted in slight, though significant, increase in step-trough latencies in passive-avoidance paradigm but all doses of scopolamine (0.4, 0.8 and 1.2 mg/kg) impaired performance in the twenty-four hour retention task [20].

Many authors reported memory enhancing effects of well-established cholinesterase inhibitors - galantamine, rivastigmine and donepezil on scopolamine-induced memory deficit.

Rivastigmine is a potent acetyl- and butyrylcholinesterase inhibitor approved for cognitive improvement in Alzheimer’s disease (AD) therapy. However, dose-limiting adverse effects restrict its tolerability and clinical outcomes. The results of Zhang et al. demonstrated that rivastigmine (0.75 and 2.0 mg/kg) could significantly reverse the cognitive deficit in mice induced by scopolamine (0.25 mg/kg) in passive avoidance test [2]. Donepezil also reversed scopolamine-induced deficits (3 mg/kg, s.c.) in a passive avoidance task at all doses tested, across a log-scale dose-range from 0.01-1.0 mg/kg [21, 22]. In the work of Bores and colleagues (Bores et al. 1996), a beneficial effect of galantamine was observed on behavior, by attenuating scopolamine-induced deficits in passive avoidance [23].

**Passive avoidance after repeated administration**

Antagonism of scopolamine-induced deficits in PA learning by anticholinesterase drugs shows the dominance of cholinergic influence during acquisition and consolidation phases in memory functions. The most important finding of the study of Nath et al. is that acquisition and consolidation process of learned task, rather than recall, is more susceptible to inhibition of cholinergic influence. Preferential influence of cholinergic system on acquisition and consolidation observed in the study correlates with the impairment in acquisition and consolidation of recent events and information without affecting past memory in patients of AD [24].

Our results indicated decrease in latency times in learning sessions between 1st and 5th day after drug administrations except in 4d group. The increase in latency times was observed in all groups except 4a and 4d groups on the 12th day, when memory retention test was performed. This can be explained with the weaker influence of scopolamine on the memory retention and with potential memory enhancing effect of the investigated compounds.

**Conclusion**

The compounds 4a and 4c increase the latency of entry in the dark chamber in passive avoidance after single administration in a doses which does not increase locomotor activity. The most pronounced effect is observed in the following sequence: 4c > 4a > Galantamine. The other three compounds did not show any significant change. After repeated 5-day administration the best results in passive avoidance was observed in 4d group. The increase in latency times was observed in all groups except 4a and 4d groups on the 12th day, when memory retention test was performed.
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