Effect of Piracetam, Vinpocetine and Ginkgo Biloba Extract on Antipsychotic-Induced Impairment of Learning and Memory

Abstract
Haloperidol is a classic neuroleptic drug with the known drawback that it induces motor abnormalities and impaired learning. Aim: To investigate the nootropic drugs piracetam, vinpocetine and extract of Ginkgo biloba for their ability to improve spatial memory in mice treated with haloperidol. Methods: Spatial memory was assessed by means of the Morris water maze (MWM) test. The effects of piracetam (50, 150 or 300 mg/kg, i.p.), vinpocetine (1, 2 or 4 mg/kg) and Ginkgo biloba extract (25, 50 or 150 mg/kg) were studied on working memory in mice treated with haloperidol (2 mg/kg, i.p.) to induce cognitive impairment [25]. The drugs were either co-administered with haloperidol or given 30 min before haloperidol administration. Results: The administration of haloperidol resulted in a significant increase in the time taken to locate a submerged test platform (latency). The time taken to locate the submerged platform was reduced dose-dependently by piracetam co-administered with haloperidol or given 30 min prior to the antipsychotic drug. Vinpocetine co-administered with haloperidol failed to improve cognitive performance, but vinpocetine at 4 mg/kg administered 30 min before haloperidol markedly reduced the time to locate the submerged platform. Mice treated with Ginkgo biloba extract showed worsening of their performance on the water maze test. Conclusion: Piracetam and vinpocetine, but not Ginkgo biloba extract, alleviate haloperidol-induced impairment of learning and memory in the MWM test. Human studies are needed to establish whether piracetam as well as vinpocetine may prove of value in improving cognition in patients treated with classic antipsychotic drugs.

Key words
spatial memory – haloperidol – nootropics – mice

Souhrn
Haloperidol je klasické neuroleptikum, které vyvolává motorické abnormity a poruchy učení. Cíl studie: Zhodnotit schopnost nootropic piracetamu, vinpocetinu a Ginkgo biloba zlepšit prostorovou paměť myší léčených haloperidolem. Metodologie: Prostorová paměť byla hodnocena ve Morrisově vodním bludišti (Morris water maze, MWM). Měřena byla účinnost piracetamu (50, 150 nebo 300 mg/kg i.p.), vinpocetinu (1, 2 nebo 4 mg/kg) nebo Ginkgo biloba (25, 50 nebo 150 mg/kg) na pracovní paměť myší léčených haloperidolem (2 mg/kg, i.p.) podávaným k vyvoláni kognitivní poruchy [25]. Léčivé látky byly buď podávány současně s haloperidolem, nebo 30 minut před podáním haloperidolu. Výsledky: Podání haloperidolu vedlo k významnému prodloužení latence při hledání ponouknuté plošiny. Piracetam podaný současně s haloperidolem, nebo 30 minut před antipsychotikem zkracoval v závislosti na dávce latenci při hledání ponouknuté plošiny. Vinpocetin podaný současně s haloperidolem kognitivní výkon zlepšil, ale vinpocetin 4 mg/kg podaný 30 minut před haloperidolem zvýraznil zkratil latenci při hledání ponouknuté plošiny. U myší léčených Ginkgo biloba došlo ke zhoršení výkonu ve vodním bludišti. Závěr: Piracetam a vinpocetin, avšak nikoli Ginkgo biloba, zlepšují poruchy učení a paměti vyvolané haloperidolem v Morrisově vodním bludišti. K potvrzení případné užitečnosti piracetamu a vinpocetinu při zlepšování kognice u pacientů léčených klasickými antipsychotiky je zapotřebí provést studie na lidských jedincích.

Key words
prostorová paměť – haloperidol – nootropika – myši

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Introduction

Memory, or the retention of learned information, is fundamental to human beings. Memory impairment such as that occurring in normal aging or in pathological conditions, e.g. Alzheimer’s disease, is a serious medical and social problem. At present, few drugs are available for the treatment of memory disorders. These include the pyrrolidine derivative piracetam [1], the synthetic vincamine derivative vinpocetine [2] and standardized extracts of Ginkgo biloba leaves [3]. Although these drugs belong to distinct and different chemical classes, they share the term “nootropic”, introduced by Giurgea in 1973 [4], to indicate a category of drugs that enhances memory, facilitates learning and protects memory processes against conditions that tend to disrupt them.

Piracetam, a pyrrolidine derivative (2-oxo-1-pyrrolidinone acetamide), was the first nootropic (from the Greek noos ‘mind’ and tropos, ‘growth, movement towards’) drug to be introduced into clinical practice. The drug has been shown to facilitate learning and to prevent the development of amnesia under various experimental conditions [1]. Piracetam enhanced recovery from aphasia after stroke [5] and improved cognitive function in the elderly [6] and after coronary artery bypass [7]. The drug reversed hippocampal membrane alterations in Alzheimer’s disease [8] and inhibited the lipid-destabilizing effect of the amyloid peptide Abeta C-terminal fragment [9].

Vinpocetine (vinpocetine-ethyl apovincaminate), is a synthetic derivative of the alkaloid vincamine, an extract of the flower. Vinpocetine is widely used to improve cerebral glucose uptake [2,10]. In patients with ischemic stroke and mild cognitive impairment, vinpocetine favorably influenced the cognitive status in patients with chronic hypoperfusion [11]. The drug prevented scopolamine- and hypoxia-induced impairment of passive avoidance retention in rats [12] and improved short-term memory processes in patients with flunitrazepam-induced impairment of memory [13].

Standardized extracts of the leaves of Ginkgo biloba (EGb 761) are widely used as cognitive and memory enhancers in the cerebral insufficiency that occurs during normal aging or that arises out of vascular or degenerative dementias, but with variable outcome [14,15]. Extracts of Ginkgo biloba contain 24% ginkgo-flavone glycosides and 6% terpenoids (ginkgolides, bilobalide). The herb has been shown to possess antioxidant and free radical scavenging activities [16], anti-inflammatory [17], vasodilatory [18] and rheological [19] properties. Extracts of Ginkgo biloba may possess amyloid precursor protein lowering capacity as well [20].

Cognitive impairment in schizophrenia is frequent. Spatial working memory or short-term place memory is impaired in schizophrenia and the effect of antipsychotics on cognition in patients with schizophrenia is an important issue that generates considerable debate. Studies have suggested worsening of memory tasks associated with the use of the typical antipsychotic haloperidol in healthy volunteers [21–23] and in schizophrenic patients [24] compared with patients treated with atypical neuroleptics such as risperidone, olanzapine, and ziprasidone, which improved cognition in several studies [25]. Researchers found remarkably reduced psychomotor performance in the haloperidol-treated group of schizophrenic patients compared with patients treated with atypical neuroleptics [24]. Procedural learning was also found to be poorer in haloperidol-treated patients than in normal control subjects, while no difference could be observed between olanzapine-treated patients and normal control subjects [23]. The view that atypical antipsychotics improve memory in patients with schizophrenia is, however, not supported by all studies. Short-term administration of olanzapine, and not of haloperidol, impeded several aspects of psychomotor function and verbal memory in healthy volunteers [26]. Not all domains of cognition seem to be equally affected by different antipsychotics and risperidone, olanzapine, and haloperidol did not improve social cognition in schizophrenia [27]. Cognitive improvement associated with the administration of antipsychotic medication may be a manifestation of improvement in general cortical information processing [28]. Other studies, however, have suggested that improvements of cognition in schizophrenia treated with second-generation antipsychotic medications might reflect simple practice effects (i.e. exposure, familiarity, and/or procedural learning), while medication effects on cognition remain modest [29]. In any event, there is clearly a need to treat patients with schizophrenia with cognition-enhancing medications.

In experimental animals, haloperidol, which blocks D2 dopamine receptors in the striatum, causes impairment of memory retention in terms of latency time to find the original location of the platform in a water-maze task [30]. Haloperidol as well as clozapine impaired the acquisition process and consolidation processes respectively in step-through test. Both drugs impaired spatial learning function in mice in the water maze task [31]. The present study was therefore designed to investigate the effect of the memory enhancing drugs piracetam, vinpocetine and Ginkgo biloba extract in terms of their ability to improve spatial memory in mice treated with haloperidol.

Materials and methods

Animals

Swiss male albino mice of 20–22 g body weight were employed. Standard laboratory food and water were provided ad libitum. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). Equal groups of six mice each were used in all experiments.

Cognitive testing

The Morris water maze (MWM) was employed to test spatial learning and memory. The MWM is a paradigm that requires the mice to use spatial memory to find a hidden platform just below the surface of a pool of water, and to remember its location from a previous trial [32]. The mice must therefore use distal cues to effectively locate the platform. Accurate navigation is rewarded by escape from the pool. The maze consisted of a glass tank narrowed to 20 cm wide, 40 cm in height, 70 cm in length, filled to a depth of 21 cm with water maintained
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to find the platform (in seconds) was assessed with a stopwatch.

Drugs
Haloperidol (Kahira Pharm and Chem. Co., Cairo, ARE), vinpocetine (Vinporal, Amrya. Pharm. Ind., Cairo, ARE), piracetam (Nootropi, Chemical Industries Development; CID, Cairo, ARE), Ginkgo biloba extract (EMA Pharm. Co., Cairo, ARE). All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use. The doses of drugs used in the study were based upon the human dose after conversion to that for mice, after conversion tables by Paget and Barnes [34]. The dose of haloperidol used in the study (2 mg/kg) was chosen on the basis of similar studies by Terry et al [35,36]. The dose would be expected to achieve comparable (and therapeutically relevant) D2 receptor occupancy values in vivo (i.e., in the range 65–80%) and based on the fact that haloperidol is metabolized over three times faster in rats than in humans.

Results
Effect of nootropics on haloperidol-induced memory impairment
Spatial memory was tested in the Morris water maze test. Haloperidol substantially impaired water maze performance. The time taken to find the escape platform (latency) was significantly delayed by haloperidol (2 mg/kg, i.p.). Animals given piracetam co-administered with haloperidol or given 30 min prior to the antipsychotic drug showed significantly shorter latencies, which indicated that learning had occurred immediately (Fig. 1). All groups exhibited learning and improved their performance with training, analyzed as the time taken to reach the hidden platform (F = 11.58; p < 0.0001). There was also a significant effect of treatment (F = 136.96; p < 0.0001) and a significant treatment × session interaction (F = 2.87; p = 0.002).

Post-hoc comparison indicated significantly shorter latencies for location of the hidden platform on the part of all treated groups in trials 1, 3 and 3 compared with the haloperidol control group. Furthermore, in trial 2, the group treated with the highest dose of piracetam showed significantly shorter latencies than all other treatment groups.

Vinpocetine co-administered with haloperidol failed to improve cognitive performance on water maze test, but vinpocetine at 4 mg/kg administered 30 min before haloperidol markedly reduced the latencies for location of the submerged platform (Fig. 2). There was a significant drug effect (F = 105.34; p < 0.0001), but insignificant session effect (F = 1.99; p = 0.143) or treatment × session interaction (F = 0.434; p = 0.96).

In trial 1, post-hoc comparisons indicated significantly shorter escape latencies for mice co-treated or pretreated

Fig. 1. Effect of piracetam on haloperidol-induced (2 mg/kg, i.p.) impairment of learning and memory of mice in the Morris water maze test.

Piracetam was given intraperitoneally (i.p.) either at the time of haloperidol administration or 30 min before haloperidol administration. The latency (in seconds) to find the platform was determined for control and drug-treated groups. Each mouse was given three trials (columns 1, 2, 3) (trial 1: reference memory or acquisition trial (first column), trials 2 and 3: working memory or retrieval trial (second and third column) which used the same platform location and start. Data represent mean values (± SE) of 6 mice per group. Statistically significant differences vs. haloperidol control group are indicated by asterisks. For other group differences, see text.
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With 4 mg/kg vinpocetine compared with other treatment groups. In trial 2, co-treatment with 2–4 mg/kg vinpocetine as well as pretreatment with 1–4 mg/kg vinpocetine was associated with significantly shorter escape latencies compared with haloperidol control. Meanwhile, co-treatment or pretreatment with 4 mg/kg vinpocetine resulted in significantly shorter escape latencies compared with other treatment groups. Thus the highest dose of vinpocetine resulted in significantly shorter escape latencies in all trials compared with the haloperidol-control group.

Mice treated with Ginkgo biloba extract showed worsening of their performance on the water maze test with markedly increased time to find the hidden platform (Fig. 3). Repeated ANOVA mea-

Fig. 2. Effect of vinpocetine on haloperidol-induced (2 mg/kg, i.p.) impairment of learning and memory of mice in the Morris water maze test.

Vinpocetine was given intraperitoneally (i.p.) either at the time of haloperidol administration or 30 min before haloperidol administration. The latency (in seconds) to find the platform was determined for control and drug-treated groups. Each mouse was given three trials (columns 1, 2, 3) (trial 1: reference memory or acquisition trial (first column), trials 2 and 3: working memory or retrieval trial (second and third column) which used the same platform location and start. Data represent mean values (±SE) of 6 mice per group. Statistically significant differences vs. haloperidol control group are indicated by asterisks. For other group differences, see text.

Fig. 3. Effect of Ginkgo biloba extract on haloperidol-induced (2 mg/kg, i.p.) impairment of learning and memory of mice in the Morris water maze test.

Ginkgo biloba extract was given intraperitoneally (i.p.) either at the time of haloperidol administration or 30 min before haloperidol administration. The latency (in seconds) to find the platform was determined for control and drug-treated groups. Each mouse was given three trials (columns 1, 2, 3) (trial 1: reference memory or acquisition trial (first column), trials 2 and 3: working memory or retrieval trial (second and third column) which used the same platform location and start. Data represent mean values (±SE) of 6 mice per group. Statistically significant differences vs. haloperidol control group are indicated by asterisks. For other group differences please refer to the text.
The current study investigated the effect of the nootropic drugs piracetam, vinpocetine and Ginkgo biloba extract on haloperidol-induced impairment of learning and memory in the water maze test. Favorable effects were observed after piracetam and the highest dose of vinpocetine examined, but not after Ginkgo biloba extract. Piracetam administered either as co-treatment or pretreatment markedly alleviated the impairment of learning and memory induced by haloperidol. The drug appeared to be nearly equally effective at all doses examined (50, 100 and 300 mg/kg). Piracetam has proved effective in several models of amnesia. It has been shown to facilitate learning and retrieval of information and protect the brain from physical and chemical intoxication [45]. It reverses the amnesia induced by scopolamine, electroconvulsive shock and morphine as well as that caused by hypoxia [46,47]. Piracetam has negated the amnesic effect of 6-hydroxydopamine and restored to control values the noradrenaline level in the frontal cortex and hippocampus [48]. It has been shown to increase cortical and striatal monoamines [49,50], which might underlie its cognitive enhancing properties.

The latency of haloperidol-treated mice in location of the submerged platform in the MWM test was also reduced by 4 mg/kg vinpocetine given either at the same time as haloperidol administration or 30 min prior to haloperidol. Other researchers have shown that vinpocetine prevents scopolamine- and hypoxia-induced impairment of passive avoidance retention in rats [12]. Vinpocetine and its main metabolite cis-apovincaminic acid protects against NMDA-induced neurotoxicity in a rat model of dementia. Behavioral deficits, such as impaired recognition of novel objects and spatial learning performance in the Morris maze, lesion size and microglia activation, have been markedly alleviated by vinpocetine and cis-apovincaminic acid [51]. The drug improved flunitrazepam-induced impairment of memory in healthy volunteers treated with flunitrazepam [13], and led to significant cognitive improvement in elderly patients with chronic cerebral dysfunction [52] as well as in patients with ischemic stroke and mild cognitive impairment [11]. Other researchers found no clear benefit demonstrated in patients with acute ischemic stroke [53]. The drug reduced accumulation of reactive oxygen species and blocked the inhibition of the mitochondrial respiratory chain complexes II–III and IV induced in cells by toxic concentrations of Aβ peptides [54]. In vitro, vinpocetine in a 1–50 µM/ml concentration range protected against glutamate excitotoxicity in primary cortical neuronal cultures [55]. The drug binds to the peripheral-type benzodiazepine receptor involved in the mitochondrial transition pore complex [56] and reduces the decrease in mitochondrial inner membrane potential induced by glutamate exposure [55]. These properties are likely to mediate the beneficial influence of vinpocetine in cerebrovascular disease. In patients with Alzheimer’s disease, vinpocetine up to 60 mg per day, however, failed to improve cognition or overall functioning [57].

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mory task [67]. The effects of Ginkgo bi-loba extract on spatial memory have been ascribed to cholinergic activity and per-haps partly to a histaminergic mechanism [68], to increased synaptic plasticity [69] and to increased level of 5-hydroxytryptamine in the hippocampus [70]. In this study, the administration of Ginkgo bi-loba extract even made impairment of cognitive performance induced by haloperidol worse in the water maze test, lead- ing to higher latencies of location for the platform.

In summary, the current study showed that piracetam and vinpocetine, but not the herbal remedy Ginkgo bi-loba extract, alleviated the impairment of learning and memory induced by the typical anti-psychotic haloperidol. Further studies are warranted in patients with schizophrenia to ascertain whether Ginkgo bi-loba extract might be suitable for improving cog-nitive function in patients on antipsy-chotic drug therapy and to evaluate the utility of using piracetam and vinpocetine to improve cognition in patients treated with classic antipsychotic drugs.

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EFFECT OF PIRACETAM, VINPOCETINE AND GINKGO BILOBA EXTRACT


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