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

Účinnost piracetamu, vinpocetinu a Ginkgo biloba na poruchy učení a paměti vyvolané antipsychotiky.

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.

Souhrn
Haloperidol je klasické neuroleptikum, které vyvolává motorické abnormity a poruchy učení. Cíl studie: Zhodnotit schopnost nootropik piracetamu, vinpocetinu a Ginkgo biloba zlepšit prostorovou paměť myší léčených haloperidolem. Metodologie: Prostorová paměť byla hodnocena v 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 vyvolání kognitivní poruchy [25]. Léčivé látky byly bud podávány současně s haloperidolem, nebo 30 minut před podáním haloperidolu. Výsledky: Podání haloperidolu vedlo k významnému prodloužení latence při hledání ponořené plošiny. Piracetam podaný současně s haloperidolem nebo 30 minut před antipsychotikem zkracoval v závislosti na dávce latenci při hledání ponořené plošiny. Vinpocetin podaný s haloperidolem zkrátil výkon nezlepšil, ale vinpocetin 4 mg/kg podaný 30 min před haloperidolem zkratil latenci při hledání ponořené plošiny. Ginkgo biloba došlo ke zhoršení výkonnosti ve vodním bludišti. Závěr: Piracetam a vinpocetin, avšak nikoli Ginkgo biloba, zlepšují poruchy učení a paměti vyvolané haloperidolem v Morrisově vodním bludišti. K potvrzování případné užitečnosti piracetamu a vinpocetinu při zlepšování kognice u pacientů léčených klasickými antipsychotiky je zapotřebí provést studie na lidských jedincích.

Key words
spatial memory – haloperidol – nootropics – mice

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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 enhance memory, facilitates learning and protects memory processes against conditions that tend to disrupt them.

Piracetam, a pyrrolidine derivative (2-oxo-1-pyrrolidine acetic acid), 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 42-terminal fragment [9].

Vinpocetine (vinpocetine-ethyl apovincaminate), is a synthetic derivative of the alkaloid vincamine, an extract of the lesser periwinkle (Vincia minor), a wildflower. Vinpocetine is widely used to improve cognitive function in patients with cerebrovascular disease in consideration of its ability to increase cerebral blood flow, which in turn increases regional 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...
at 25 °C. The glass escape platform was hidden from sight, submerged 1 cm below the surface of the water at the end of the tank [33]. The effect of piracetam (50, 150 or 300 mg/kg, i.p.), vinpocetine (1, 2 or 4 mg/kg) or Ginkgo biloba extract (25, 50 or 150 mg/kg) on working memory was studied in mice treated with haloperidol (2 mg/kg, i.p.) to induce cognitive impairment [30]. Drugs were either co-administered with haloperidol or given 30 min before haloperidol administration in order to see whether a difference exists or not when cognitive enhancers are given prior to the drug-produced memory impairment. Mice rapidly learn to swim directly to the escape platform and climb out. Once a mouse reached the platform, it remained there for 15 sec (trial 1; reference memory or acquisition trial). At the end of each trial, the mouse was towel-dried, returned to its home cage (where a heat lamp was available), and 3 min elapsed before the next trial (trials 2 and 3; working memory or retrieval trial), using the same platform location and start position as trial 1. The latency 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.

**Statistical Analysis**

Data are expressed as mean ± SEM. The data were analyzed by one way ANOVA and by repeated measures (session x treatment) ANOVA, followed by Duncan’s multiple range test, using SPSS software (SAS Institute Inc., Cary, NC). A probability value of less than 0.05 was considered statistically significant.

**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 x 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 x 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.
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-

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**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.

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**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.
sures indicated a significant treatment ef-
fect, $F = 92.5; p <0.0001$, but insignificant
trial effect, $F = 1.98; p = 0.144$ or treat-
ment x trial interaction, $F = 1.74; p = 0.64$.

Post-hoc comparisons revealed a sig-
nificant increase in escape latencies in
trial 1, 2 and 3 on co-treatment with
150 mg/kg ginkgo and by pretreatment
with 50 or 150 mg/kg of Ginkgo biloba
eXtract compared with other treatment
groups. In addition, pretreatment with
150 mg/kg of Ginkgo biloba extract was
associated with significant increase in es-
cape latencies in trial 2 and 3 compared with other treatment groups.

Discussion
In the current study, haloperidol sub-
stantially impaired the water maze per-
formance of mice. Both the acquisition
and retention of information were impai-
red by the drug. The dopaminergic sys-
tem plays an important role in memory
processes [37,38]. Studies have sugges-
ted that D2-like receptors have a greater
contribution to make than D1-like recep-
tors to both spatial working memory and
object-location associative memory [39].
The D2-dopaminergic system is involved
in the induction of basolateral amygdala
to long-term potentiation of the dentate
gyrus [40]. Haloperidol impaired spatial
working memory performance and plan-
nning ability in healthy volunteers [21,22]
and worsened recent autobiographical
memory scores of patients with Alzhe-
imer’s disease [41]. In experimental ani-
mals haloperidol, and to lesser extent
olanzapine and risperidone, impaired working memory performance [42].
In the current study, the dose of haloperidol
used was shown to impair locomotor ac-
tivity in a previous task [43] and drug in-
fluence on motor activity cannot be ruled
out, but the water maze test is primarily
test of cognition (spatial learning/me-
ory) [44]. Other workers have reached the conclusion that the negative effects
of haloperidol in the water maze task are
likely to be memory-related [35].

The current study investigated the effect of the nootropic drugs piraceta-
tam, vinpocetine and Ginkgo biloba ex-
tact on haloperidol-induced impairment of
learning and memory in the water
maze test. Favorable effects were obser-
v ed after piracetam and the highest dose
of vinpocetine examined, but not after
Ginkgo biloba extract. Piracetam admi-
nistered either as co-treatment or pre-
treatment markedly alleviated the impair-
ment of learning and memory induced
by haloperidol. The drug appeared to be
nearly equally effective at all doses exami-
ned (50, 100 and 300 mg/kg). Piracetam
has proved effective in several models
of amnesia. It has been shown to facil-
tate learning and retrieval of informa-
tion and protect the brain from physical
and chemical intoxication [45]. It rever-
ses the amnesia induced by scopolamine,
electroconvulsive shock and morphine as
well as that caused by hypoxia [46,47].
Piracetam has negated the amnesiastic
effect of 6-hydroxydopamine and resto-
red to control values the noradrenaline
level in the frontal cortex and hippocam-
pus [48]. It has been shown to increase
cortical and striatal monoamines [49,50],
which might underlie its cognitive enhan-
cing 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 re-
searchers have shown that vinpocetine
prevented scopolamine- and hypoxia-in-
duced impairment of passive avoidance
retention in rats [12]. Vinpocetine and its
main metabolite cis-apovincaminic acid
protects against NMDA-induced neuroto-
xicity in a rat model of dementia. Behavio-
ral deficits, such as impaired recognition
of novel objects and spatial learning per-
formance in the Morris maze, lesion size
and microglia activation, have been mar-
kedly alleviated by vinpocetine and cis-
apovincaminic acid [51]. The drug impro-
ved flunitrazepam-induced impairment of
memory in healthy volunteers treated
with flunitrazepam [13], and led to sig-
nificant cognitive improvement in elderly
patients with chronic cerebral dysfunction
[52] as well as in patients with ischemic
Other researchers found no clear bene-
fit demonstrated in patients with acute
ischemic stroke [53]. The drug reduced
accumulation of reactive oxygen species
and blocked the inhibition of the mito-
chondrial respiratory chain complexes II–III
and IV induced in cells by toxic concentra-
tions of Aβ peptides [54]. In vitro, vinpo-
cetine in a 1–50 µM/ml concentration
range protected against glutamate excito-
toxicity in primary cortical neuronal cul-
tures [55]. The drug binds to the peripheral-
type benzodiazepine receptor involved in
the mitochondrial transition pore complex
[56] and reduces the decrease in mito-
chondrial inner membrane potential in-
duced by glutamate exposure [55]. These
properties are likely to mediate the bene-
ential influence of vinpocetine in cerebro-
vascular disease. In patients with Alzhe-
imer’s disease, vinpocetine up to 60 mg
day, however, failed to improve co-
gnition or overall functioning [57].

Ginkgo biloba extract extracts are wi-
dely used to treat memory disorders,
although their value is not yet clear. In
one study in patients with mild cognitive
impairment, about half of the patients trea-
ted with Ginkgo biloba extract experi-
ced an improvement in memory and their
ability to concentrate, as well as a decre-
ase in symptoms of forgetfulness [58]. In
a randomized clinical trial, Ginkgo biloba
extract neither altered the risk of pro-
gression from normal to clinical dementia
nor protected against decline in memory
function [59]. In another study, Ginkgo
biloba extract at 120 mg twice a day was
not effective in reducing either the overall
incidence rate of dementia or Alzheimer’s
disease incidence in elderly individuals
with normal cognition or those with mild
cognitive impairment [60]. Ginkgo bi-
loba extract (120 mg per day) had no sig-
ificant effect on a wide range of cogni-
tive abilities, executive function, attention
and mood in healthy older adults and in
young adults [61]. In healthy young vo-
lunteers, Ginkgo biloba extract adminis-
tration improved quality of memory 1
and 4 h post-dosing, but decreased the speed
of attention task performance [62]. In rats
subjected to chronic restraint stress, Ginkgo
biloba extract decreased hippocampal neu-
ronal loss and cognitive dysfunction [63]
and improved spatial memory [64]. It was
also effective in reducing, at least partially,
both cognitive impairments and hippo-
campal damage after transient forebrain
ischemia in rats [65]. Others have sugges-
ted that Ginkgo biloba extract does not
enhance short-term working memory or
long-term memory reference, but rather
promotes learning of spatial information
[66] and that Ginkgo biloba extract does
don’t offer any continued beneficial ef-
fects in an already-learned working me-
mory task [67]. The effects of Ginkgo biloba extract on spatial memory have been ascribed to cholinergic activity and perhaps 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 biloba extract even made impairment of cognitive performance induced by haloperidol worse in the water maze test, leading to higher latencies of location for the platform.

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

References

EFFECT OF PIRACETAM, VINPOCETINE AND GINKGO BILoba EXTRACT


Tento článek podle autorskému zakonu a jeho vyzvání je možné s příslušným prohlášením: www.csnn.eu/prohlasi/


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