THE CENTRAL HISTAMINE LEVEL IN RAT MODEL OF VASCULAR DEMENTIA

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The histaminergic system plays an important role in memory and learning. Deficient histaminergic transmission in the human brain in vascular dementia (VD) has been suggested. To get a better insight into the problem, a rat model of VD based on permanent bilateral occlusion of the common carotid arteries (BCCAO) leading to chronic cerebral hypoperfusion was used. Prior to the BCCAO, male Wistar rats underwent 7 days training and only those animals that positively passed the holeboard memory test were chosen for the study. The rats which were operated on were injected i.p. daily for 6 days with either a monoamine oxidase B inhibitor - PF9601N (40 mg/kg), an acetylcholinesterase inhibitor - tacrine (3 mg/kg), a histamine H3 receptor blocker - DL76 (6 mg/kg) or saline. The first retest (R1) was performed one week after the surgery while each subsequent test was 5–7 days apart. The rats were euthanized 2 or 4 weeks following the operation. The concentration of brain histamine (HA) and the activity of histamine metabolising enzymes were measured using current procedures. The BCCAO drastically increased latency and run time (p<0.001) 54±30 vs 3.4±1.2 and 268±18 vs 74±9, respectively, and affected working memory rather than reference memory as measured by the 1st retest (R1). Treatment with either PF9601N or tacrine seems to exert a positive effect on working memory. This tendency disappeared after the drug treatment stopped. Latency and run time, although they improved in R2-R4, never attained the preoperative values. The brain tissues from rats treated with PF9601N showed only 15% and 50% of untreated rat MAO B and MAO A activity, respectively, despite the drug administration having been discontinued for 3 weeks. Other drugs examined did not influence MAO enzymes. Neither did histamine N-methyltransferase activity show changes related to BCCAO nor to the treatments. The hypothalamic HA concentration was significantly reduced after BCCAO: 1.13±0.1 vs 1.91±0.16. Noteworthy, the rats treated with PF9601N or DL76 had brain HA levels not significantly different from their intact counterparts. The rat vascular dementia model supports deficiency in histaminergic system in VD.

Key words: histamine, histamine H3 antagonist, monoamine oxidase inhibition, permanent bilateral occlusion of the common carotid arteries, vascular dementia

INTRODUCTION

Histamine plays an important role in regulation of a wide range of physiological and pathophysiological processes and acts via four distinct histamine receptor subtypes: H1-H4 (1-3). In the brain histamine is synthesized in neurons of the tuberomammillary nucleus located in the posterior hypothalamus, which project their fibers to almost all regions of the brain (4). The histaminergic neurons are involved in the modulation of sleep-wakefulness cycle, hormonal secretion, appetite control, thermoregulation, sexual activity and emotional behaviour amongst others (1). Moreover, the histaminergic system plays an important role in cognitive function, memory and learning, and participates in central nervous system disorders leading to neurodegenerative diseases (3, 5, 6).

The mechanisms underlying neurodegenerative diseases, such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and vascular dementia (VD), have not yet been fully explained (7-8).

There is evidence that they share several common features (5) and are associated with progressive loss of a selective kind of neurons in the brain.

AD is characterized by the gradual loss of cholinergic neurons, resulting in cognitive and behavioral disorders and by the presence of morphological alterations in the brain, i.e. protein aggregations: neurofibrillary tangles containing hyperphosphorylated tau protein and the senile plaques mainly formed by beta-amyloid peptide (7, 10).

PD results from the death of dopaminergic neurons in the substantia nigra and striatum. Early in the course of the disease, movement-related symptoms appear, i.e. shaking, rigidity, slowness of movement and difficulty with walking. In the advanced stages of the disease, the impairment of cognitive function and behavioural problems as well as dementia may arise (8, 11, 12).

VD is the second most common cause of dementia after Alzheimer’s disease, and is associated with cognitive
deterioration caused by the occlusion of cerebral arteries and brain damage (9, 13).

Etiopathogenic mechanisms leading to neurodegenerative disorders (AD, PD, stroke-related brain damage) also include oxidative stress, cytotoxicity of reactive oxygen species, mitochondrial dysfunction and apoptosis (14-17).

To date, the specific etiology of neurodegenerative diseases remains unclear and current treatment for them mainly affects the symptoms of disease.

According to the cholinergic hypothesis, therapeutic approaches for the treatment of Alzheimer’s disease are focused on increasing cholinergic transmission and acetylcholinesterase (AChE) - the acetylcholine degrading enzyme - is the main pharmacological target. Therefore, AChE inhibitors are conventional drugs for the treatment of AD and have beneficial effects on the cognitive, functional and behavioural symptoms of the disease (10, 18, 19). The classical AChE inhibitors (e.g. tacrine, donepezil, rivastigmine or physostigmine) have similar efficacy with different tolerability profile (18) and also inhibit butyrylcholinesterase (BuChE). BuChE has a similar structure to AChE but its physiological role is unclear (20). It has been reported that BuChE may participate in detoxification of several chemicals in CNS as well as in pathogenesis of AD by the promoting of transformation of beta-amyloid peptide (21-23).

The first drug approved by the Food and Drug Administration’s (FDA) for treatment of Alzheimer’s disease was tacrine (24). At therapeutic effective doses, AChE inhibitors have many adverse side effects, such as nausea, vomiting, diarrhoea, abdominal pain, weight loss, anorexia (18) and hepatotoxicity associated with serum alanine aminotransferase (ALT) elevation in up to 50% of patients (25, 26). It was also shown that tacrine inhibits histamine N-methyltransferase (HMT), the main histamine metabolizing enzyme in the brain (10, 27, 28).

The treatment of PD is mainly based on dopamine replacement therapy with carbipoda, levodopa, dopamine agonists, monoamine oxidase type B inhibitors (MAO B), catechol-O-methyltransferase inhibitors and anticholinergic drugs (8, 12, 29).

Therefore, new effective therapeutic strategies that can treat or prevent the progression of neurodegenerative diseases are needed. At present, many different chemical compounds are subjects of intense experimental and clinical studies. New approaches in the treatment of dementia, focus on a novel series of non-amphetamine selective MAO B inhibitors (11, 14, 30-32) and H1 receptor antagonists (6, 33-35).

PF9601N [(N-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamino], the acetylenic tryptamine derivative devoid of amphetamine-like properties, was synthesized by Cruces et al. (30). It is a highly potent and selective MAO B inhibitor - about 50 and 5 times more selective than deprenyl and rasagiline, respectively, without a ‘cheese effect’ resulting from MAO A inhibition (36). The compound exerts protective effects in different experimental models of PD (31, 32, 37-39) and cell culture models of the oxidative stress (11, 14, 40). Taking into account all the facts, PF9601N is a good candidate for use in the prevention and therapy of PD and other neurodegenerative diseases.

The histamine H1 receptor was first described in 1983 by Arrang et al. (41) as a presynaptic autoreceptor controlling histamine biosynthesis and release. In addition, the H1 receptor acting as a heteroreceptor modulates the release of other neurotransmitters, e.g. serotonin, noradrenaline, dopamine and acetylcholine (42-44). Considering the high expression of H1 receptors in parts of the brain that are associated with cognitive function (hippocampus, basal ganglia, cortical areas) and their ability to regulate the release of key neurotransmitters, the H1 receptors antagonists would have great therapeutic potential in neurodegenerative diseases (3, 6, 33, 34). Since 1998, when Ganellin et al. (45) described the new non-imidazole histamine H1 receptor ligand, this class of compounds has been intensively studied for distinct clinical applications (35, 46-48).

The latest strategies for the treatment of neurodegenerative diseases focus on the development of multifunctional drugs, i.e. potentially proognitive hybrid compounds based on histamine H1 receptor antagonists with potency of HMT inhibition (33, 49, 50), multipotent cholinesterase and MAO inhibitors (51), as well as cholinesterase inhibitors with the ability to interfere with both formation and aggregation of the beta-amyloid peptide (52-53).

A variety of animal models of human dementia have been used to examine the behavioural and neuronal changes that occur in the diseased central nervous system (54-56).

In the present study, to get some information on the histaminergic system, a rat model of BD, based on the permanent bilateral occlusion of the common carotid arteries (BCCAO) leading to chronic cerebral hypoperfusion, was used (55, 57, 58). The effect of PF9601N and DL76 (1-[3(4-tert-butylophenoksy) propyl]piperidine), which are MAO B inhibition and histamine H1 receptor antagonist respectively, on deteriorated cognitive function caused by BCCAO using holeboard memory test (59) was examined. Tacrine, an acetylcholinesterase inhibitor, was administered as a reference drug.

MATERIAL AND METHODS

**Animals**

Male Wistar rats weighing 180–250 g at the beginning of the experiments were used for the study. The experimental procedures were undertaken according to EU directives and local ethical regulations. The rats were housed in standard cages with liquid and food available *ad libitum* with a reversed 12-h light-dark cycle with light off at 7 a.m. The psychometric tests and pharmacological treatments were carried out in the dark phase of the cycle. During the drug treatment rats were kept in metabolic cages (Tecniplast Gazzada, Italy). All the experiments were performed on pre-selected rats, *i.e.* the animals which successfully completed the holeboard memory tests (habituation and training phases). Schematic representation of the experimental procedure is shown in Fig. 1.

**Psychometric assessment (holeboard memory test)**

To measure the speed of learning and memory capacity in rats, the holeboard memory test was used (59). The testing area of the holeboard apparatus contains 16 holes in a 4x4 array. The holes are 20 cm apart and filled with plastic cups (2.5 cm deep, 3 cm in diameter) for sweetened cereal food pellets. Some were spilled on the floor to exclude the influence of smell (olfactory stimuli) on the search for food. The rats had to collect pellets in specified periods of time.

The test consists of 3 phases: habituation, training and retests. During the habituation and training phases and for one day before the retests, food was restricted for the rats to enhance their motivation to search for it (water was available *ad libitum*).

The habituation phase was for 4 consecutive days. Each hole in the board was baited with 50 mg of cereals. The trial started when the rat entered the testing area and it was stopped after 10 min or earlier, *i.e.* the time necessary to collect all food pellets. The monitoring included the time and the number of visits and revisits to the baited holes. Rats which found at least 14 of 16 pellets on the 4th day, qualified for the training phase. The ones with poorer results were excluded from further experiments (1st exclusion criterion).
Training phase started 3 days after habituation and continued for 7 consecutive days with a pause on the 5th and 6th day. The rats were trained to collect pellets from 4 holes: A1, B3, C2 and D4 (Fig. 2). During one training session, four trials were carried out for each animal. The maximum time given to collect 4 food pellets was 5 min. There were intervals of about 1 min between the trials for cleaning and baiting A1, B3, C2 and D4 holes with pellets. Apart from the time of performance, the registration included latency time (time between the beginning of the trial and the first hole visit) and the number of visits and revisits to the baited and empty holes. Based on these parameters, two distinct memory functions - working and reference memory - were evaluated.

Working memory ratio (WM) was presented as a percentage of all visits to the baited set of holes that had been supplied with food (calculated as the number of food rewarded visits divided by the number of visits and revisits to the baited set of holes). Reference memory ratio (RM) was expressed by the number of visits to the baited set of holes as a percentage of the total number of visits to all holes (calculated as the number of visits and revisits to the baited set of holes divided by number of visits and revisits to all holes). The parameters were evaluated based on results of the 7th day of training. Only rats with WM ratio >50% and RM ratio >40% qualified for the surgical procedure (BCCAO). Animals with poor results were excluded as non-intelligent.

The first retest (R1) was performed one week after the surgery, while each subsequent test was 5–7 days apart, with a new order of baited holes: A4, B2, C3, D1 (rotated by 90° as compared with the training phase) (Fig. 2). The course for the retests, as well as the registered parameters, were the same as during the training phase.

Bilateral common carotid occlusion (BCCAO)

Bilateral common carotid arteries were occluded as previously described by ketamine hydrochloride (50 mg/kg, i.m., Biowet Pulawy, Poland) and xylazine (5 mg/kg, i.m., Biowet Pulawy, Poland) anaesthetics. To prevent respiratory distress, the rats were also administered atropine sulfate (0.1 mg/kg, i.m., Polfa Warszawa, Poland). The common carotid arteries were carefully separated from surrounding tissues, including the vagus nerve and ligated with coated Vicryl (R). Plus antibacterial/Polyglactin 910 3/0 absorbable surgical suture (Ethicon, Johnson & Johnson, UK), approximately 1 cm inferior to the origin of the external carotid artery. The control rats were subjected to the same surgical procedure without occlusion of the arteries.

Pharmacological treatment

Starting the day after BCCAO the rats were treated daily with either monoamine oxidase B inhibitor, N-(2-propylnyl)-2-(5-benzyl-1H-indolyl) methylamine (PF9601N, 40 mg/kg, i.p., dissolved in 4% N,N-dimethylformamide) or acetylcholinesterase inhibitor, 9-amino-1,2,3,4-tetrahydropyridine hydrochloride (tacrine, 3 mg/kg, i.p., dissolved in 0.9% NaCl), or histamine H3 receptor antagonist, 1-[3(4-tert-butylophenox)propyl]piperidine (DL76, 6 mg/kg, i.p., dissolved in 0.9% NaCl) or physiological saline. The injections were continued for 6 consecutive days.

PF9601N and DL76 were a gift from prof. M. Űnèzeta (Department of Biochemistry and Molecular Biology, Autonomous University Barcelona, Spain) and prof. K. Kiec-Könonowicz (Department of Technology and Biotechnology of Drugs, Jagiellonian University, Cracow, Poland), respectively. Tacrine was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation and biochemical evaluations

The rats were euthanized after the 3rd - 4th retest, i.e. 2 or 3 weeks following the operation. The brain was quickly removed from the skull; the hypothalamus and the rest of the brain were dissected. Tissue samples were immediately frozen in liquid nitrogen and kept at -70°C until assayed. The cerebral histamine concentration was measured by radioimmunoassay (ImmunoTech, Beckaman Coulter Company). Histamine N-methyltransferase activity was determined radioenzymatically according to Taylor and Snyder (60) by measurements of radioactive N-tele-methylhistamine formed in a transmethylation reaction catalysed by the enzyme, as previously described (61). S-adenosyl-L-(methyl-14C)-methionine was used as a donor of methyl group.

MAO A and MAO B activities were estimated in cerebral homogenates with radioassays using serotonin (fine conc. 200 µM) and β-phenylethylamine (fine conc. 20 µM), as well as specific inhibitors - clorgyline and deprenyl (0.3 µM each), respectively (62). The enzyme activities are expressed as pmol/min/mg protein. Acetylcholinesterase (ACHE) was analysed with an AChE fluorescent activity kit (Arbor Assays, USA).

Statistical analysis

The results are expressed as means ± S.E.M. Statistical analysis was performed by using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The efficacy of training in the holeboard apparatus was assessed by the One-way ANOVA, followed by the Newman-Keuls Multiple Comparison Test. Behavioral results were evaluated by Two-way ANOVA and Bonferroni Multiple Comparison Test. The biochemical data were analyzed by One-way ANOVA, followed by the Newman-Keuls Test. P value of 0.05 or less was considered significant.
RESULTS

In compliance with the rules described in Material and Methods, rats that had found less than 14 out of 16 pellets on the last day of habituation phase had to be withdrawn from further testing (1st criterion for exclusion). In our study, this amounted to roughly 30% of the animals. Training on the holeboard apparatus gradually reduced latency and the total time needed to complete the trial and markedly improved working (WM) and reference (RM) memories. As presented in Fig. 3, the best results were registered between the 4th and 7th day. On the 7th day of training phase, rats with values less than or equal to 50% or 40% for working or reference memory ratio, respectively, did not qualify for the next stage of examinations (2nd criterion for exclusion).

### Table 1. Time parameters from the holeboard memory test: PF9601N treatment. The values are means ±S.E.M. Two-way ANOVA and Bonferroni multiple comparison test: **p<0.01 or ****p<0.0001 vs. Training – 7th day; *p<0.05 or **p<0.01 vs. R1.

<table>
<thead>
<tr>
<th>Parameter/Group</th>
<th>Before BCCA0</th>
<th>Retest</th>
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<tbody>
<tr>
<td></td>
<td>Training – 7-th day</td>
<td>R1</td>
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<tr>
<td>Time (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCCA0</td>
<td>86.3 ± 8.8</td>
<td>234.7 ± 25.9***</td>
</tr>
<tr>
<td>BCCA0+PF9601N</td>
<td>95.8 ± 8.0</td>
<td>250.5 ± 17.5***</td>
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<tr>
<td>Latency (s)</td>
<td></td>
<td></td>
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<tr>
<td>BCCA0</td>
<td>2.3 ± 0.3</td>
<td>44.5 ± 14.1**</td>
</tr>
<tr>
<td>BCCA0+PF9601N</td>
<td>4.1 ± 1.9</td>
<td>25.8 ± 7.3</td>
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</table>

### Table 2. Time parameters from the holeboard memory test: DL76 and tacrine treatment. The values are means ±S.E.M. Two-way ANOVA and Bonferroni multiple comparison test: *p<0.05, **p<0.01 or ****p<0.0001 vs. Training – 7th day; *p<0.01 or ****p<0.0001 vs. R1.

<table>
<thead>
<tr>
<th>Parameter/Group</th>
<th>Before BCCA0</th>
<th>Retest</th>
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<tbody>
<tr>
<td></td>
<td>Training – 7-th day</td>
<td>R1</td>
</tr>
<tr>
<td>Time (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCCA0</td>
<td>69.8 ± 0.1</td>
<td>254.7 ± 29.7***</td>
</tr>
<tr>
<td>BCCA0+DL76</td>
<td>73.8 ± 3.2</td>
<td>283.0 ± 12.7***</td>
</tr>
<tr>
<td>BCCA0+tacrine</td>
<td>67.3 ± 4.5</td>
<td>280.5 ± 11.4***</td>
</tr>
<tr>
<td>Latency (s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCCA0</td>
<td>1.3 ± 0.1</td>
<td>80.9 ± 48.2</td>
</tr>
<tr>
<td>BCCA0+DL76</td>
<td>1.3 ± 0.3</td>
<td>57.1 ± 43.2</td>
</tr>
<tr>
<td>BCCA0+tacrine</td>
<td>1.1 ± 0.1</td>
<td>96.6 ± 45.8</td>
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</table>
Effect of bilateral occlusion of the common carotid arteries and pharmacological treatment on the memory parameters of operated rats

The BCCAO drastically increased latency 20–60-fold and run time by about 170–260% in the operated rats, as measured by the first retest (R1; Table 1 and 2; Time: Two-way ANOVA and Bonferroni multiple comparison test, *p<0.0001). The surgery affected working memory significantly more than reference memory (Fig. 4 and 5). Seven days after the operation (R1) WM had declined by about 40%, while RM had declined by less than 20%, compared to the results obtained on the last day of training phase. In BCCAO group (without any treatment), WM ratio was significantly lower, compared to the results recorded on 7th day of training (Fig. 4; Two-way ANOVA and Bonferroni multiple comparison test, *p<0.01). Treatment with any of the compounds used - PF9601N or tacrine or DL76 - appears to exert a positive effect on measured memory functions. In PF9601N and tacrine treated groups WM and RM ratio were maintained at the same levels which had been registered on the 7th day of training (R1: Fig. 4 and 5, respectively), whereas DL76 administration improved improved working memory rather than reference memory (Fig. 5). This tendency disappeared when the treatment was discontinued. During R1, WM ratio in PF9601N and tacrine treated rats was significantly higher compared to BCCAO group without any treatment (Fig. 4 and 5; Two-way ANOVA and Bonferroni multiple comparison test, *p<0.05). In BCCAO group None of the drugs changed the time parameters.

During R2 - R4 all the animal groups studied achieved similar results. Latency and run time, although they improved in R2 - R4, did not attain the preoperative values (Table 1 and 2). With regard to BCCAO group, WM ratio registered in R2 was significantly raised compared to R1 (Fig. 5; Two-way ANOVA and Bonferroni multiple comparison test: *p<0.05). In BCCAO group None of the drugs changed the time parameters.

Biochemical examination

The BCCAO by itself did not influence brain MAO A and MAO B activity (Table 3). The brain tissues from BCCAO rats treated with PF9601N showed significantly reduced enzyme activities, despite the drug administration having been

**Table 3.** The effect of BCCAO and treatment with PF9601N, tacrine or DL-76 on MAOs activities. The values are mean ±S.E.M for 5-9 rats. RB – rest of brain, HTH – hypothalamus. One way analysis of variance and Student-Newman-Keuls Test, ***p<0.001 or ****p<0.0001 vs. Control; ——p<0.0001 vs. BCCAO; —p<0.0001 vs. BCCAO+tacrine; —p<0.01 or ——p<0.0001 vs. BCCAO+DL76.

<table>
<thead>
<tr>
<th>Group</th>
<th>MAO A (pmol/min/mg protein)</th>
<th>MAO B (pmol/min/mg protein)</th>
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<tbody>
<tr>
<td></td>
<td>RB</td>
<td>HTH</td>
</tr>
<tr>
<td>BCCAO</td>
<td>960.20 ± 68.07</td>
<td>947.80 ± 88.66</td>
</tr>
<tr>
<td>BCCAO+PF9601N</td>
<td>508.44 ± 44.20</td>
<td>490.22 ± 60.93</td>
</tr>
<tr>
<td></td>
<td><strong>p&lt;0.0001 vs. BCCAO</strong></td>
<td><strong>p&lt;0.0001 vs. BCCAO</strong></td>
</tr>
<tr>
<td>BCCAO+tacrine</td>
<td>1010.00 ± 30.60</td>
<td>995.20 ± 48.03</td>
</tr>
<tr>
<td>BCCAO+DL76</td>
<td>860.80 ± 41.75</td>
<td>989.80 ± 36.61</td>
</tr>
<tr>
<td>Control (Intact)</td>
<td>899.50 ± 29.95</td>
<td>1075.83 ± 99.40</td>
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**Fig. 4.** Memory parameters from the holeboard memory test: PF9601N treatment. The values are means ±S.E.M. Two-way ANOVA and Bonferroni multiple comparison test: **p<0.01 vs. Tr. 7th day (Training – 7th day), *p<0.05 vs. BCCAO.**
Role in controlling the level of central histamine and also, as autoreceptors, play a very important role in the modulation of cognitive processes. As already observed, more than 20 years ago, that centrally administered histamine facilitates memorisation (66). Later studies provided convincing evidence that the activation of histaminergic neurons enhances cognition and memory (67). As mentioned in the introduction, the histamine H3 receptors, as autoreceptors, play a very important role in controlling the level of central histamine and also, as heteroreceptors, regulate the release of other neurotransmitters, including ACh. According to earlier reports, histamine may also directly modulate the activity of cholinergic neurons via H1 receptors and indirectly - by H2 receptors. Via the H2 receptors, histamine may inhibit dopaminergic and GABAergic transmission and in this way increase or decrease ACh release (65, 68). On the other hand, histamine release may be regulated by inhibitory M1 muscarinic receptors located at the endings of the histaminergic neurons (68-71). It has been also demonstrated that histamine improved memory deficits evoked by scopolamine (72).

Morphological studies have reported neurofibrillary degeneration of the tuberomamillary nucleus, the region of origin of histamine neurons, in patients with AD. It has been suggested that degeneration of histamine neurons may contribute to the cognitive decline of AD (67).

The level of activity of this histaminergic system in AD remains unknown. However, in the cerebrospinal fluid (CSF) in patients with AD, a higher level of tele-methylhistamine (t-MeHA) has been observed (67, 73). The t-MeHA is the main cerebral histamine metabolite and its concentration reflects histamine turnover and histaminergic system activity (74). It should be mentioned that, besides neurons, the brain histaminergic system includes also mast cells and microglia. Therefore, the increased level of t-MeHA in CSF may result from the activation of microglia caused by neuroinflammatory processes occurring in AD (67). It has also been shown in a mouse model of AD, that memantine (NMDA-receptor antagonist approved for the treatment of AD) activated histamine neurons, as shown by the 60% increase of brain t-MeHA, and the therapeutic efficacy of this drug may be partly depend on activation of histaminergic system (73).

DISCUSSION

It is well established, that cerebral cholinergic transmission plays a leading role in the modulation of cognitive processes. As well as that there is a close relationship between the cholinergic and histaminergic systems in learning and memory and other brain activities (2, 6, 63-65). It was already observed, more than 20 years ago, that centrally administered histamine facilitates memorisation (66). Later studies provided convincing evidence that the activation of histaminergic neurons enhances cognition and memory (67). As mentioned in the introduction, the histamine H3 receptors, as autoreceptors, play a very important role in controlling the level of central histamine and also, as

discontinued for 3 weeks. The compound blocked the MAO B enzyme almost totally in the rest of the brain and decreased its activity more than 2-fold in hypothalamus (One-way analysis of variance and Student-Newman-Keuls Test, p<0.0001). In both parts of brain which were examined, only 40% of untreated /control rat MAO A activity was noted (One-way analysis of variance and Student-Newman-Keuls Test, p<0.0001). Other examined drugs did not influence MAO enzymes. At 2–3 weeks after the operation, histamine concentration was significantly reduced after BCCAO nor to the treatments (data not shown). Hypothalamic histamine concentration was significantly reduced after BCCAO: 1.13±0.10 vs. 1.91±0.16 (One-way analysis of variance and Student-Newman-Keuls Test, p=0.01) (Fig. 6). Such an effect was maintained in the tacrine treated BCCAO group (p<0.05). Noteworthy, the rats treated with PF9601N or DL76 have brain HA levels not significantly different from their intact counterparts (3 weeks after drugs discontinuation).

Fig. 5. Memory parameters from the holeboard memory test: DL76 and tacrine treatment. The values are means ±S.E.M. Two-way ANOVA and Bonferroni multiple comparison test: *p<0.05 vs. R1, #p<0.05 vs. BCCAO.

<table>
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<tr>
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<th>BCCAO</th>
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<tr>
<td>WM</td>
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<tr>
<td>R1</td>
<td>65±4.3</td>
<td>64±4.5</td>
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<td>R2</td>
<td>42±10.6</td>
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<td>R3</td>
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<tr>
<td>RM</td>
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<th>BCCAO</th>
<th>BCCAO + DL76</th>
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To get better insight into the role of histamine in pathogenesis of dementia, in the presented study, we used a rat model of vascular dementia. Permanent, bilateral common carotid artery occlusion in the rat is a well-characterized valuable tool for assessing early neuronal dysfunction and the effects of potential neuroprotective therapies in neurodegenerative disorders. BCCAO leads to chronic cerebral hypoperfusion, which reflects neurodegenerative processes in human dementia and is associated with cognitive decline (55). In humans, VD, in addition to AD, represents the most common form of dementia (75).

Prior the BCCAO, in order to eliminate other causes of random memory disorders, rats underwent an initial holeboard memory test, according to Lanert and Hoyer (59). Only the animals, which positively passed this first behavioural evaluation were entered into further experiments. As is shown on Fig. 3, 7-days training on the holeboard apparatus significantly increased short- and long-term memory, designated respectively as working (WM) and reference (RM) memory, and decreased time parameters, i.e. total time and latency. The results obtained on the 7th day allowed us to choose “intelligent” rats with a large capacity for learning.

As measured by subsequent retests, BCCAO resulted in a dramatic increase in time parameters and decline of memory function (Fig. 4 and 5). These disturbances may be in part a result of brain ischemia and neuronal degeneration caused by surgery.

Post mortem, in BCCAO group, a significant decrease in brain histamine concentration compared to intact rats was observed (Fig. 6). Therefore, cognitive dysfunction after BCCAO may in part also result from deficits in the cerebral histaminergic system. These findings confirmed deficient histaminergic transmission in the human brain in VD (5) and suggest that histamine may be implicated in the pathogenesis of dementia (67, 73). Considering this suggestion, as well as certain neurological and behavioural similarities between AD and VD, memantine will be possibly effective in the treatment of VD (multi-infarct dementia) (75).

The hypoperfusion evoked by BCCAO only has a transient character, and due to compensatory collateral circulation, cerebral blood flow normalizes at 8 weeks (58). This fact may explain the partial recovery of cognitive function in later retests (i.e. R2 - R4; Fig. 4 and 5).

All the tested compounds (tacrine or PF9601N or DL76) had positive effect on deteriorated memory function caused by BCCAO as well as protect cerebral histamine deficits. This last is supported by more recent data, which show that PF9601N used in a single dose of 5 mg/kg b.w., i.p., evoked in rat brain a significant rise in both tele-methylhistamine (4.38±0.67 vs. untreated: 1.98±0.29, p<0.001) and histamine (1.60±0.44 vs. untreated: 0.88±0.25, p<0.001).

It should be noted that none of the groups subjected to any treatment achieved pre-operative results in memory parameters.

Cognition deficits and memory loss after BCCAO may be partly due to cholinergic dysfunction in the brain. Accordingly, the decrease of ACh level in the striatum, cortex and hypothalamus has been observed up to 4 months post operation. Interestingly, 1 month post BCCAO, a significant decline in ACh was noted only in the striatal tissue (76). These neurochemical data indicate that BCCAO leads to progressive and time-dependent neuronal degeneration.

The present results support the possibility that the impairment of cognitive function after BCCAO may depend on central cholinergic deficits. In tacrine treated rats, both working and reference memory were maintained at the same levels which were registered on the 7th day of training (Fig. 5; R1). However, this positive tendency was only observed during the first retest and disappeared after stopping therapy. Tacrine belongs to a reversible class of AChE inhibitors (18). Following oral administration, the drug is rapidly and well absorbed (peak plasma concentrations: 0.5 to 3 hours). It has a mean bioavailability about 17% and a short pharmacokinetic half-life (maximum up to 4 hours) (24, 77). For the same reason, 3 weeks post-treatment, we did not record any decrease in cerebral AChE and HMT activities (data not shown). Thus, the clinical efficacy provides a continuous drug administration, 4 times a day in a total dose of 40–80 mg.

The improvement of memory deficit in BCCAO rats treated with tacrine has also been noted by Murakami et al. (78). Moreover, it has been reported that administration of acetylcholinesterase inhibitors produced cognitive benefit in other experimental models of dementia. Tacrine was effective in procedures directly based on cholinergic deficits (79, 80) and rivastigmine enhanced memory function disturbed by colchicine (56).

The deficit in cholinergic neurotransmission, a characteristic of Alzheimer’s disease, has also been postulated to contribute to the cognitive disturbances of vascular disease of the brain in humans. Cholinesterase inhibitors, such as donepezil, may therefore be a promising treatment for patients with VD (13).

Fig. 6. Histamine concentration in the CNS; the effects of BCCAO and treatments. TACR-tacrine, PF – PF9601N. The values are means ±S.E.M. The number of rats is given in parentheses. One way analysis of variance and Student-Newman-Keuls Test, *p<0.05 or **p<0.01 vs. Control; ’p<0.05 vs. BCCAO+PF.
Based on the reports regarding the therapeutic efficacy of non-imidazole H1 receptor antagonist in cognitive disorders (35, 46-48), we decided to test DL76, a newly synthesized compound (81). Very recently, the compound was the subject of Pharmacodynamic and pharmacokinetic evaluations (82). Earlier studies have shown its good affinity for the human H3 receptor stably expressed in CHO-K1 cells (81). In our experimental paradigm, DL76 elicited a positive effect on deteriorated memory function caused by BCCAO and influenced working rather than reference memory (Fig. 5, R1).

As is clearly demonstrated in this study, the brain tissue from rats treated with PF9601N showed significantly reduced MAOs activities, even 3 weeks after cessation of therapy (Table 3). The compound blocked almost totally MAO B as well as decreasing MAO A activity by 60%. These results support earlier reports (based on in vitro evaluation) that PF9601N is a highly potent and selective, suicide MAO B inhibitor (36). F9601N was shown to have neuroprotective effects in some experimental models of PD; it reduced the loss of dopaminergic neurons evoked by different neurotoxins: MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (32, 38), 6-OHDA (6-hydroxydopamine) (31, 37) and MPP+ (1-methyl-4-phenylpyridinium) (39), both in vitro and in vivo experiments. In the presented study, the compound expressed a positive effect on cognitive function after BCCAO (Fig. 4, R1). This observation suggests the possibility of using the drug in the treatment of other neurodegenerative diseases, apart from Parkinson’s disease. The inhibition of MAOs by PF9601N was also associated with an increased level of dopamine and serotonin in rat brains (to be published). While serotonin is exclusively metabolized by MAO A, dopamine is a substrate for both forms of enzyme. However, it has previously been suggested that the neuroprotective activity of PF9601N depends rather on its antioxidant and antiapoptotic properties than MAO B inhibition (39, 40, 83, 84).

In conclusion, treatment with all used compounds (PF9601N, DL76 and tacrine) seems to exert a positive effect on cognitive disturbance caused by BCCAO and the rat vascular dementia model supports deficiency in histaminergic system in VD. All tested compounds protected memory function, as the memory parameters recorded during therapy (R1) have been at the same level as these registered on 7th day of training. Evidently, the working memory was significantly more affected by BCCAO than the reference one.

Despite intensive research on neurodegenerative disorders and their etiology for decades, current treatment for them remains still symptomatical and only transiently effective. Accordingly, thorough understanding of the mechanisms leading to neuronal degeneration is critical for improving clinical treatment and developing novel therapeutic strategies.

The presented study warrants further research on therapeutic efficacy of newly-synthesized multifunctional compounds.

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