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Original article

Chronic cerebral hypoperfusion-induced memory impairment and hippocampal long-term potentiation deficits are improved by cholinergic stimulation in rats

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ABSTRACT

Background: Chronic cerebral hypoperfusion (CCH) can induce the accumulation of reactive oxygen species, which leads to oxidative damage, neuronal injury, and central cholinergic dysfunction in vulnerable regions of the brain, such as the hippocampus and cerebral cortex. These effects can lead to significant cognitive impairments in clinical populations of vascular dementia (VaD). The present studies aimed to investigate the role of the cholinergic system in memory functions and hippocampal long-term potentiation (LTP) impairments induced by CCH in rats.
Methods: Male Sprague Dawley rats were subjected to permanent bilateral occlusion of common carotid arteries (PBOCCA) or sham surgery. Then, PBOCCA rats received *ip* injections with, either vehicle (control group), the muscarinic receptor agonist oxotremorine (0.1 mg/kg), or the acetylcholinesterase inhibitor physostigmine (0.1 mg/kg). Cognitive functions were evaluated using a passive avoidance task and the Morris water maze test. In addition, hippocampal LTP was recorded *in vivo* under anaesthesia.
Results: The PBOCCA rats exhibited significant deficits in passive avoidance retention and spatial learning and memory tests. They also showed a suppression of LTP formation in the hippocampus. Oxotremorine and physostigmine significantly improved the learning and memory deficits as well as the suppression of LTP in PBOCCA rats.
Conclusions: The present data suggest that the cholinergic system plays an important role in CCH-induced cognitive deficits and could be an effective therapeutic target for the treatment of VaD.

Introduction

Dementia is a syndrome related to a general decline in cognition and memory functions. Alzheimer's disease (AD), the most common form of dementia, manifests by the accumulation of amyloid plaques and neurofibrillary tangles in the brain [1]. Vascular demencia (VaD), the second most prevalent type of dementia after AD, results from various types of vascular-related diseases. The incidence rate of VaD has been reported to be 6–12 cases per 1000 people over 70 years of age annually [2,3]. Risk factors, such as advanced age, hypertension, high blood cholesterol, and arteriosclerosis, cause moderate cerebral hypoperfusion and ischemic brain injuries, which lead to progressive decline in cognitive and memory functions, as observed in VaD and AD [4,5].

Chronic cerebral hypoperfusion (CCH) is a condition of reduced cerebral blood flow in the brain, which can induce the accumulation of reactive oxygen species (ROS) and the deprivation of glucose and oxygen, which are vital for normal cellular functioning and the survival of brain tissue. A sustained reduction of cerebral blood flow may initiate a cascade of neuropathological events, such as microglial activation, neurodegeneration, and cholinergic dysfunction that are thought to contribute to the cognitive impairment observed in AD and VaD [6,7]. In our previous studies, we employed a PBOCCA rat model to study the time course of behavioural functions after CCH. Our data have shown that PBOCCA effectively impaired cognitive functions without affecting basic motor function over the course of 4 weeks period. Thus, this model provides a valuable approach to discover the potential neuroprotective strategies against CCH-induced learning and memory impairments [8].

Numerous pharmacological studies have supported a crucial role of acetylcholine (ACh) in cognition by demonstrating that drugs known to interfere with cholinergic transmission impair

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cognitive performance [9–11], while cholinergic agonists such as physostigmine, an acetylcholinesterase (AChE) inhibitor, or oxotremorine, a muscarinic receptor agonist, can improve cognitive functions or reverse cognitive deficits in humans and non-human species [12–15]. ACh is easily metabolized into choline and acetate by the enzyme AChE and other non-specific esterases. Hence, inhibition of these enzymes will provide a potential way of increasing the amount of ACh in the synapse and helping to restore cholinergic function in AD patients. Physostigmine, an AChE inhibitor is known to augment the amount of ACh available in the synaptic cleft. Another approach to cholinergic therapy is the targeting of cholinergic receptors. Direct receptor activation may improve cognitive performance. In addition, degeneration of basal forebrain cholinergic neurons (BFCN), which widely innervate the hippocampus and neocortex, has been linked to cognitive impairments occurring in VaD and AD [16,17].

LTP is a long-lasting enhancement of synaptic strength induced by repetitive high frequency stimulation of the pre-synaptic terminal. Thereby, the Schaffer collateral of the CA3 area of the hippocampus is stimulated and extracellular field potentials can be recorded from the pyramidal cell layer of CA1. In contrast, long term depression (LTD) describes the long-lasting decrease in synaptic strength following repetitive low frequency stimulation [18,19]. LTP is the leading candidate for activity-dependent synaptic mechanisms which underlie learning and memory functions. Moreover, cholinergic transmission has been suggested to play an important role in the modulation of synaptic plasticity [20,21]. Even though LTP is found in several other regions of the brain which are associated with certain forms of memory, LTP in the hippocampus is the most studied form of synaptic plasticity in the mammalian brain [18,22].

To date, a potentially causal relationship between CCH, cholinergic system dysfunction, and neurobehavioural- and LTP abnormalities has not been established. In the present study, we evaluated CCH-induced memory impairments, hippocampal LTP suppression, and assessed the role of the cholinergic system in these impairments.

Materials and methods

Animals

Male Sprague Dawley (SD) rats, 7 weeks old and weighing between 200–250 g, were obtained from the breeding colony of the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia (USM). They were housed five rats per cage and maintained at a constant temperature on a standard 12:12 light/dark cycle with lights on at 7 a.m.. Food and water were given *ad libitum*. The rats were 12 weeks old at the beginning of the experiment. All procedures performed in studies involving animals were conducted with the approval of the Animal Ethics Committee, USM, with the reference number USM/Animal Ethics Approval/2014/ (92) (569).

Surgery

PBOCCA surgery was performed in the rats as described previously [8]. Briefly, the rats were anaesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) *ip*. In rats randomly chosen for PBOCCA (n = 50), the common carotid arteries were exposed *via* a ventral midline incision, carefully separated from their sheaths and the vagus nerves, and permanently doubly ligated with 5/0 silk suture. The similar surgery procedure was employed to sham operation rats (n = 14) without ligation. The survival rate of animal was approximately 90% following the PBOCCA surgery. Behavioural test were started on day 28 after the surgery with comparable bodyweight in all groups: Sham + saline, 299.57 ± 3.34 g, PBOCCA + Saline, 295.29 ± 4.79 g, PBOCCA + Oxo, 299.14 ± 3.74 g, PBOCCA + Physos, 297.43 ± 5.13 g. Only animals that completed the whole test procedure in good health were considered for data analysis.

Treatment conditions

The drugs used were oxotremorine, a nonselective muscarinic receptor agonist, and physostigmine, an AChE inhibitor (Sigma, USA). The drugs were dissolved in physiological (pH 7.0) saline. The rats were randomly divided into four groups for behavioural tests and four groups for the LTP experiment. Physiological (pH 7.0) saline was used as vehicle in this study. Group 1: sham + saline (n = 7); Group 2: PBOCCA + saline (n = 7); Group 3: PBOCCA + oxotremorine 0.1 mg/kg (n = 7); and Group 4: PBOCCA + physostigmine 0.1 mg/kg (n = 7). Different group of animals were used for behavioural studies and LTP experiment. In the behavioural studies, rats were subjected to Passive avoidance task and, subsequently to Morris water maze. The drug injection volume was 1 ml/kg, which was administered immediately after the training for behavioural tests (Passive avoidance task: single injection, Morris water maze: repeated injections on 5 consecutive days) and after the surgery and 30 min before the baseline recording for electrophysiological studies (single injection). The selected dose of oxotremorine (0.1 mg/kg) and physostigmine (0.1 mg/kg) are based on the dosage that produced memory-enhancing effects in animal models [23,24].

Passive avoidance task

The experimental protocol was performed as previously described [8]. On day 28 after the surgery, an acquisition trial was performed for habituation purpose. Herein, each rat was allowed to freely move in both light and dark compartments. During the training session on the next day, once the rat crossed into the dark compartment with all four paws, a 0.5 mA foot shock was administered for 10 s. Those rats that spent more than 100 s to cross to the dark compartment were excluded from the experiment. Twenty-four hours after the training session, a retention test was performed for memory assessment. No electric shock was applied during this test session. The step-through latency into the dark compartment on the test day was used as a retention score.

Morris water maze

The Morris water maze task was performed as previously described [8]. In brief, a circular pool of water (160 cm in diameter, 70 cm high and of 39 cm depth) that was coloured opaque by the addition of white paint to the water was maintained at 25 ± 1 °C. The pool was divided into four quadrants. A platform, 10 cm in diameter, was situated 2 cm below the surface of the water at a fixed position in one quadrant. On the first day, the rat was allowed to freely explore the pool for 60 s without an escape platform for habituation purpose. Thereafter, training sessions with a submerged escape platform were conducted for 5 consecutive days with four trials per day. Twenty-four hours after the training session, a probe trial test was performed in the absence of the platform to evaluate memory retention. The percentage time spent in the target quadrant was calculated as a measure of spatial reference memory. Visual, motor and motivational performance was assessed immediately after the probe trial using a visible platform test. In this trial, the visible platform was placed 1 cm above the water surface, and the time taken to reach the platform was recorded.

In vivo *electrophysiological recordings*

Surgery procedures

This experiment was conducted as previously described [25]. Animals were anaesthetised with urethane (Sigma Aldrich, USA) 2.0 g/kg, *ip*, in four 0.5 g/kg doses every 20 min, and an additional dose when required. The local analgesic xylocaine (5 mg/kg) was injected *sc* in the incision region. Then, the animal was placed in a stereotaxic apparatus. Two burr holes were drilled on the skull for electrode placement into the stratum pyramidale of the hippocampal CA1 (AP: -4.2 mm, ML: -3.0 mm, V: -3.0 mm) and contralateral CA3 region of the hippocampus (AP: -4.2 mm, ML: + 3.0 mm, V: -4.0 mm), respectively, according to [26]. Another two holes were drilled for screws placement in the bone overlying the frontal cortex to serve as references and ground connections for the recording electrode. The recording electrode was connected to an amplifier (AM system), allowing the signal to be amplified, filtered (0.1–500 Hz) and digitized (10 kHz) by a Powerlab/4SP system (AD Instruments, Australia) and stored in a computer using LabChart 7 software for offline analysis. A bipolar concentric microelectrode was connected to a stimulus isolator unit (ML 180 Stimulus Isolator, AD Instruments) providing a constant current output.

In vivo *electrophysiological recording*

The intensity required to produce the maximal field excitatory postsynaptic potential (fEPSP) amplitude was determined *via* input-output curves with 0.2-ms stimulation pulses delivered to the CA3 at intensities of 0.1–1.0 mA in 0.1 mA increments. The purpose of increasing the stimulation intensities was to measure any changes in basal synaptic transmission. A stimulation intensity eliciting approximately 50–60% of the maximal fEPSP amplitude was chosen from the input-output curves for the remainder of the experiment. For each experiment, a 60 min period of initial baseline fEPSP was recorded every 30 s to achieve a stable baseline. Then, a theta burst stimulation (TBS) was applied as a train of ten bursts (each burst consisting of 5 pulses at 100 Hz) with bursts repeated every 200 msec for a single train. Finally, the fEPSP was recorded every 30 s for 3 h following TBS [25,27]. At the end of the experiment, the rat's brain was removed and electrode placement was verified [26] (see: Supplementary data). Only animals with accurate placement of electrodes were considered for data analyses.

Statistics

Behavioural performance in the Morris water maze, input-output curves and time course changes in fEPSP amplitude after TBS were analysed using a two-way repeated-measure ANOVA, followed by Bonferroni *post-hoc* tests. Statistical analysis for the passive avoidance task, probe trial, visible platform and mean of the fEPSP amplitude were performed using one-way ANOVA, followed by Bonferroni *post-hoc* test. All data were expressed as the means ± SEM. Probability values less than 5% ($p < 0.05$) were considered significant.

Results

Passive avoidance task

Initially, we assessed the performance of the rats in the passive avoidance task. The time taken by the rats to step through to the dark, previously shocked-paired compartment during the retention trial is shown in Fig. 1. The PBOCCA-saline group showed significantly shorter step-through latencies compared to the sham-saline group ($p = 0.001$). Interestingly, the step-through latency increased significantly in the PBOCCA rats treated with oxotremorine ($p = 0.04$) and physostigmine ($p = 0.005$) compared to the PBOCCA-saline group. However, the step through latency among groups in the passive avoidance test on the foot-shock test day were not significantly different (p>0.05).

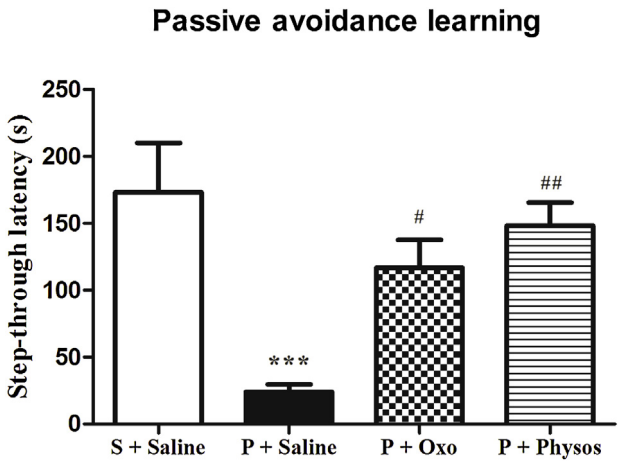

Fig. 1. Effects of oxotremorine (Oxo; 0.1 mg/kg, *ip*) and physostigmine (Physos; 0.1 mg/kg, *ip*) on memory impairment induced by bilateral occlusion of the common carotid arteries (P) in a passive avoidance task (S – sham surgery). Data are expressed as the mean ± SEM. (n = 6–7/group) $^{***}p < 0.001$ *vs.* sham-saline group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* PBOCCA-Saline group (One-way ANOVA followed by Bonferroni *post-hoc* tests).

Morris water maze

As shown in Fig. 2A, the PBOCCA-saline group displayed longer escape latencies during the acquisition phase in the Morris water maze relative to the sham-saline group. A two-way repeated measure ANOVA revealed a significant effect of drug ($F_{3,60} = 10.26$; $p = 0.001$) and test day ($F_{4,60} = 99.05$; $p = 0.001$) and also a drug x test day interaction ($F_{12,60} = 3.63$; $p = 0.001$). Data analysis revealed a statistically significant improvement of task performance in the oxotremorine and physostigmine treated groups. The PBOCCA rats that received oxotremorine had significantly shorter latencies at day 4 ($p = 0.016$) and day 5 ($p = 0.03$) than the PBOCCA-saline group. Similarly, the escape latencies were significantly decreased in the PBOCCA rats injected with physostigmine at day 3 ($p = 0.004$), day 4 ($p = 0.009$), and day 5 ($p = 0.047$) compared to the PBOCCA-saline group (Fig. 2A). These results suggest that physostigmine and oxotremorine are effective in reversing some of the behavioural deficits associated with CCH.

Probe trial performance was assessed on the sixth day, *i.e.* 24 h after the last training session. Oxotremorine ($p = 0.024$) and physostigmine ($p = 0.009$) treated groups spent significantly more time in the target quadrant compared to saline group (Fig. 2B). The visible platform version of the water maze task did not show any significant effect of drugs on performance ($p = 0.228$, Fig. 2C).

In vivo *electrophysiological recordings*

The effect of cholinergic drugs on basal synaptic transmission in the hippocampus of PBOCCA rats was assessed using input-output curves. A two-way repeated measures ANOVA performed on input-output curves demonstrated a significant effect of the drug treatment ($F_{3,108} = 8.99$; $p = 0.002$), of stimulation intensity ($F_{9,108} = 283.51$; $p = 0.001$) and a treatment x stimulation intensity interaction ($F_{27,108} = 5.08$; $p = 0.001$). As shown in Fig. 3A, the PBOCCA-saline group showed a significant depression of the fEPSP amplitude starting from 0.3 to 1.0 mA. Oxotremorine showed a significant increase of fEPSP amplitude at 0.7 mA ($p = 0.046$) and

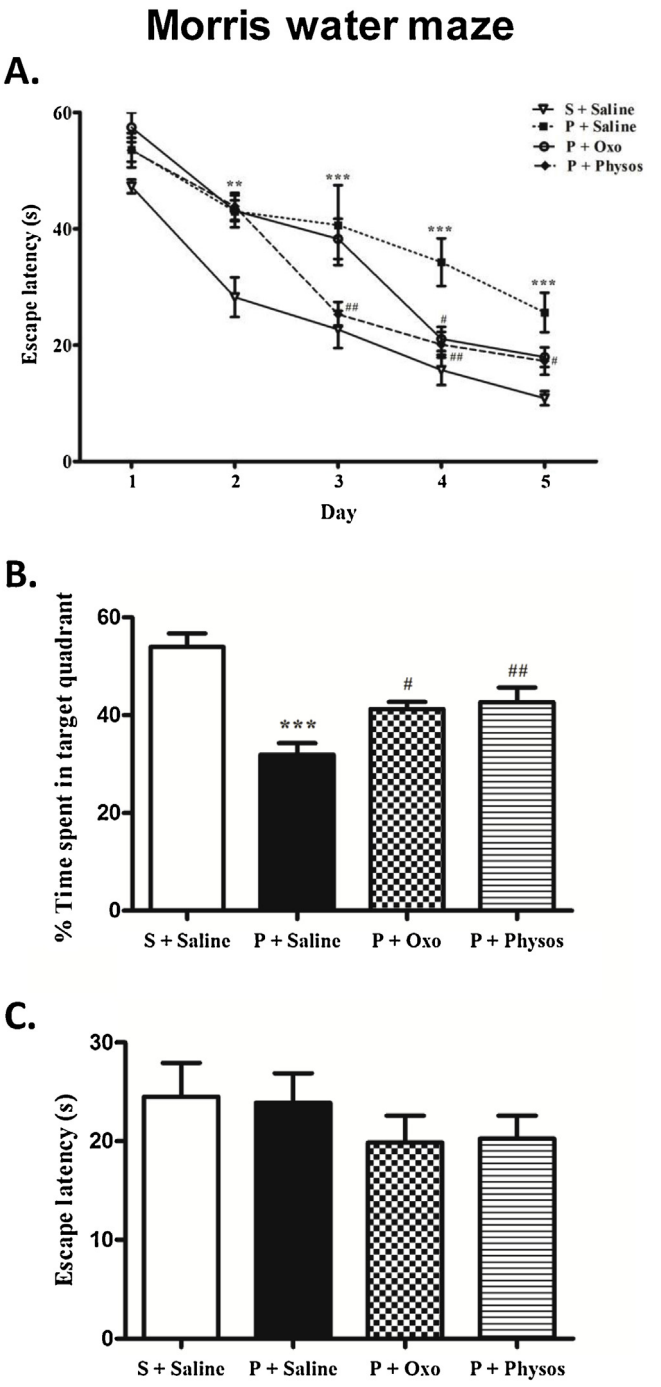

Fig. 2. Effects of oxotremorine (Oxo; 0.1 mg/kg, *ip*) and physostigmine (Physos; 0.1 mg/kg, *ip*) on the spatial learning deficits induced by bilateral occlusion of the common carotid arteries (P) in the Morris water maze (S – sham surgery). (A) Five days of training period, (B) probe trial performance, and (C) visual, motor and motivational performance. Data are expressed as the mean ± SEM. (n = 6/group). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* sham-saline group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* PBOCCA-saline group (Two-way repeated measures and One-way ANOVA followed by Bonferroni *post-hoc* test).

0.8 mA ($p = 0.022$). Physostigmine increased the fEPSP amplitude at 0.9 mA ($p < 0.034$) and 1.0 mA ($p < 0.025$) vs. the PBOCCA-saline group.

The injection of oxotremorine or physostigmine reversed the hippocampal LTP suppression in PBOCCA rats with a significant increase of maximal fEPSP amplitude to 90% and 80%, respectively.

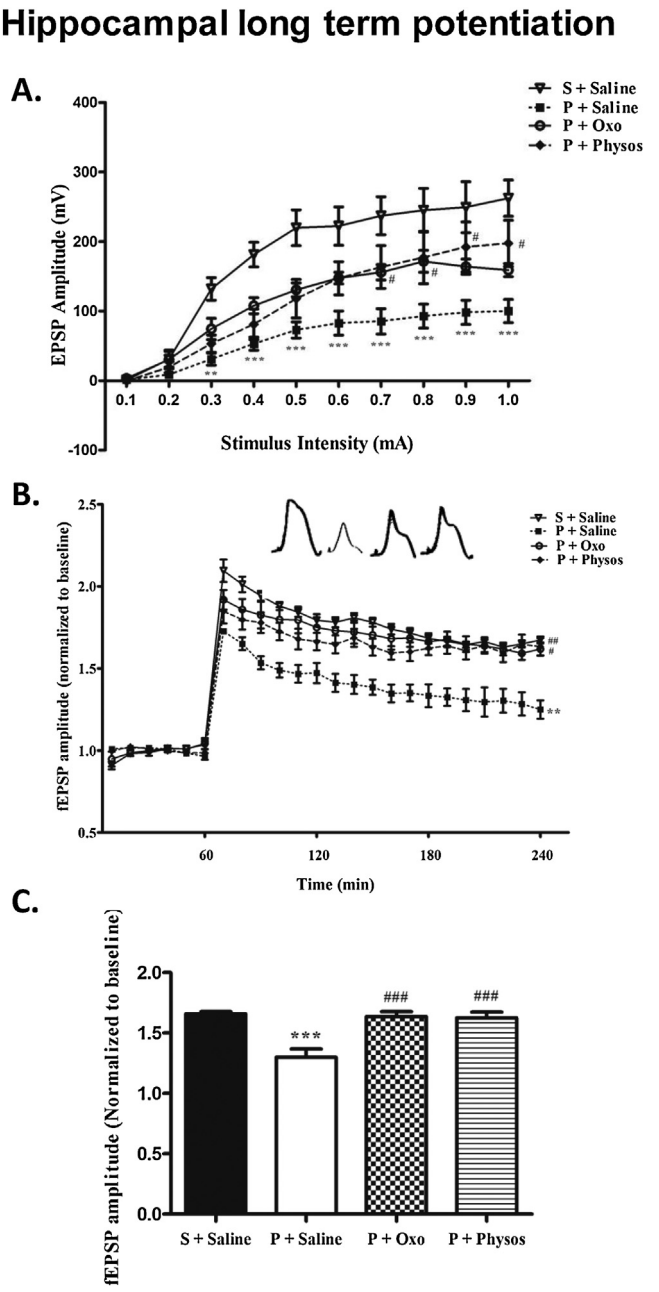

Fig. 3. Effects of oxotremorine (Oxo; 0.1 mg/kg, *ip*) and physostigmine (Physos; 0.1 mg/kg, *ip*) on LTP in the CA1 hippocampus. (A) Input/output relationship, (B) change in fEPSP amplitude before and after TBS, and (C) the mean of fEPSP amplitude for last 60 min of 3 h LTP recording following TBS. Inserts on top of graph show typical fEPSP traces of response after TBS. Data are expressed as the mean ± SEM. (n = 6/group). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* sham-saline. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs.* PBOCCA-saline (Two-way repeated measures and one-way ANOVA followed by Bonferroni *post-hoc* test).

The level of potentiation remained stable throughout the 3 h of recording in comparison to PBOCCA-saline group. A two-way repeated measures ANOVA revealed a significant effect of treatment ($F3,276 = 9.40$; $p = 0.002$), of test time ($F_{23,276} = 442.97$; $p = 0.001$) and treatment x time interaction ($F_{69,276} = 6.01$; $p = 0.001$; Fig. 3B).

The PBOCCA-saline group (1.30 ± 0.07) exhibited a significant inhibition of hippocampal LTP in comparison with the sham-saline group (1.66 ± 0.02; $p = 0.001$). In the oxotremorine (1.64 ± 0.04; $p = 0.001$) and physostigmine (1.63 ± 0.04; $p = 0.001$) treated groups the mean of fEPSP amplitude during last 60 min was

significantly increased compared to the PBOCCA-saline group. These results suggest that an enhancement of cholinergic transmission could lead to a long-lasting LTP improvement in PBOCCA rats (Fig. 3C).

Discussion

Cholinergic projections from the basal forebrain medial septal nucleus and the vertical limb of the diagonal band of Broca to the cortex and hippocampus play an important role in memory functions [28]. Cognitive decline induced by CCH is associated with the degeneration of basal forebrain cholinergic neurons [29]. Importantly, a study on the brains of VaD patients found that cholinergic activity had been compromised [30,31]. In view of this, we investigated the effects of the cholinesterase inhibitor, physostigmine and the muscarinic receptor agonist, oxotremorine, on the cognitive impairment and LTP inhibition induced by CCH in rats. We found that enhancing cholinergic activation reversed the cognitive impairments induced by PBOCCA in two learning tests. Furthermore, activation of the cholinergic system reversed CCH-induced *in vivo* hippocampal LTP suppression at the Schaffer collateral CA3-CA1 synapse.

Extensive studies have proven a pivotal role of the central cholinergic system in modulating cognitive processes in both humans and animals [32,11,28]. In addition, there is a positive correlation between cognitive deficits and activity in the cholinergic system [33–35]. In the present study, we used a passive avoidance task to evaluate the effects of physostigmine and oxotremorine on 24 h memory retention of an aversive event. Consistent with previous work, the results showed passive avoidance retention as impaired following CCH [8]. Here we demonstrate that PBOCCA rats that received physostigmine or oxotremorine exhibit a significantly increased step-through latency compared to the saline treated PBOCCA rats (Fig. 1). Thus, the present study suggests that stimulation of cholinergic activity could at least partially reverse the impaired learning and memory functions in the PBOCCA rats.

From previous studies it is known that rats subjected to CCH exhibit marked spatial reference learning and memory deficits [8,36,37]. We observed significant behavioural impairments in the PBOCCA group in a test of spatial memory. In the motivational test, there was no significant difference in escape latency between the PBOCCA and sham groups. These results indicate that the learning and memory deficit in the PBOCCA group was not due to vision impairment or motor dysfunction (Fig. 2C). On the other hand, treatment with oxotremorine ameliorated the spatial memory impairment in the PBOCCA rats only in the late phase of the training. This was evident by the reduction in escape latencies starting from day 4 during the training sessions in comparison to the PBOCCA-saline group (Fig. 2A). This observation suggests that sustained activation of muscarinic receptors may be required during the acquisition phase of a hippocampus-dependent spatial memory task. Subsequently, the reference memory, which was assessed 24 h after the last treatment in a probe trial test, was improved for PBOCCA rats having received oxotremorine after post-training. The time spent at the target quadrant was significantly increased compared to the saline group (Fig. 2B). These results strongly suggest that an activation of the central cholinergic system *via* muscarinic receptor stimulation ameliorates the cognitive impairment induced by CCH in rats.

There are some interactions between the cholinergic system and cerebral blood flow through cholinergic innervation of cerebral blood vessels. It has been reported that a treatment with cholinesterase inhibitors, such as physostigmine and eptastigmine, increases the extracellular level of endogenous acetylcholine, leading to a significant increase of cerebral blood flow in different brain regions in rats and humans [38–40]. In the present study, we found that the spatial learning deficit induced by CCH was improved after the administration of physostigmine, starting from day 3 to day 5 of the training period, as well as in probe trials when the platform was removed from the pool in the Morris water maze (Fig. 2A and B). Furthermore, physostigmine administration did not alter non-mnemonic factors such as motivation, motor, or sensory processes which were assessed during the visible platform test. These results suggest that possible mechanisms underlying the improvement of cognitive function by the cholinergic system may include the restoration of the cerebral blood flow in the PBOCCA rats.

LTP at the CA1 synapse is a well-known cellular model for exploring synaptic mechanisms underlying long-term memory in the hippocampus. The first part of the experiment involved an assessment of the effects of oxotremorine and physostigmine on basal synaptic transmission at this synapse in the hippocampus by evoked fEPSP at the CA1 region in response to increasing stimulation of the CA3 region. The basal synaptic transmission at this synapse was impaired in PBOCCA rats. However, after injection of oxotremorine or physostigmine, the synaptic transmission deficit was reversed in the PBOCCA rats. This reversal was noticed through an increase in the fEPSP amplitude at higher intensities (0.7–1.0 mA) (Fig. 3A). Therefore, it is apparent that these drugs enhance the input-output properties of the CA1 neuron. This enhancement may occur by improving the basal synaptic strength of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor-mediated transmission at CA3-CA1 synapse in the hippocampus *via* a presynaptic mechanism [19].

The *in vivo* electrophysiological recordings showed that CCH markedly suppressed the potentiation of LTP in the Schaffer collateral pathway of the hippocampus in PBOCCA rats. This observation is in line with the findings reported by other investigators [41,42]. Interestingly, both oxotremorine and physostigmine partly restored the *in vivo* hippocampal LTP suppression at Schaffer collateral CA3-CA1 synapse caused by CCH. This was evident by an increase in the levels of the fEPSP amplitude potentiation following TBS. The potentiation persisted over a period of 3 h (Fig. 3B). These data support the view that the cholinergic system is critical for LTP at the CA1 synapse [43,44]. Previous work reported a prevalent reduction in cognitive ability and a synaptic plasticity impairment in neurodegenerative-related disorders such as VaD and AD [45,46]. Recently, Wei et al. [47] reported that miR-9-5p inhibition restores LTP suppression and cholinergic deficits, which was correlated with improved learning and memory capacity in PBOCCA rats. miR-9-5p is a microRNA located on chromosome 3 of the mouse genome. Wei et al. (2017) reported that miR-9-5p was upregulated in both the serum and cerebrospinal fluid of patient with VaD and in the hippocampus and cortex of CCH rats. Reduction or inhibition of miR-9-5p rescued learning and memory performance, synaptic plasticity, dendritic spines, cholinergic neurons, oxidative stress level, and the neuronal loss induced by CCH. Thus, the restoration of hippocampal LTP may be one of the mechanisms by which oxotremorine and physostigmine produce cognitive improvements in PBOCCA rats.

Conclusion

These findings further support the involvement of the cholinergic system in counteracting the cognitive decline induced by CCH. Activation of the cholinergic system through muscarinic receptor and inhibition of cholinesterase activity may represent a potential target for the treatment of impaired cognitive function in neurodegenerative disorders such as VaD.

Conflicts of interests

The authors declare that they have no conflict of interest.

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References

[1] Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheirmer's disease. Lancet 2011;377:1019–31.
[2] Hébert R, Brayne C. Epidemiology of vascular dementia. Neuroepidemiology 1995;14:240–57.
[3] van der Flier WM, Scheltens P. Epidemiology and risk factors of dementia. J Neurol Neurosurg Psychiatry 2005;76(supplement 5):v2–7.
[4] de la Torre JC. Cardiovascular risk factors promote brain hypoperfusion leading to cognitive decline and dementia. Cardiovasc Psychiatry Neurol 2012;1–15.
[5] Polidori MC, Marvardi M, Cherubini A, Senin U, Mecocci P. Heart disease and vascular risk factors in the cognitively impaired elderly: implications for Alzheimer's dementia. Aging Clin Exp Res 2001;13(3):231–9.
[6] Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. Trends Neurosci 2005;28(4):202–8.
[7] Farkas E, Luiten PG, Bari F. Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. Brain Res Rev 2007;54(1):162–80.
[8] Damodaran T, Hassan Z, Navaratnam V, Muzaimi M, Ng G, Müller CP, et al. Time course of motor and cognitive functions after chronic cerebral ischemia in rats. Behav Brain Res 2014;275:252–8.
[9] Drachman DA, Leavitt J. Human memory and the cholinergic system: a relationship to aging? Arch Neurol 1974;30(2):113–21.
[10] Gold PE. Acetylcholine modulation of neural systems involved in learning and memory. Neurobiol Learn Mem 2003;80(3):194–210.
[11] Pepeu G, Giovannini MG. Cholinesterase inhibitors and memory. Chem Biol Interact 2010;187(1–3):403–8.
[12] Davis KL, Mohs RC, Tinklenberg JR, Pfefferbaum A, Hollister LE, Kopell BS. Physostigmine: improvement on long-term memory process in normal subjects. Science 1978;219:272–4.
[13] Baratti CM, Huygens P, Miño J, Merlo A, Gardella J. Memory facilitation with post trial injection of oxotremorine and physostigmine in mice. Psychopharmacology 1979;64(1):85–8.
[14] Baratti CM, Kopf SR. The post-training memory enhancement induced by physostigmine and oxotremorine in mice is not state-dependent. Neurobiol Learn Mem 1996;65(2):121–4.
[15] Bodick NC, Offen WW, Levey AI, Cutler NR, Gauthier SG, Satlin A, et al. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioural symptoms in Alzheimer disease. Arch Neurol 1997;54(4):465–73.
[16] Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982;217(4558):408–14.
[17] Sharp SI, Francis PT, Elliott MS, Kalaria RN, Bajic N, Hortobagyi T, et al. Choline acetyltransferase activity in vascular dementia and stroke. Dement Geriatr Cogn Disord 2009;28(3):233–8.
[18] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31–9.
[19] Malenka RC. LTP and LTD: dynamic and interactive processes of synaptic plasticity. Neuroscientist 1995;1(1):35–42.
[20] Jerusalinsky D, Kornisiuk E, Izquierdo I. Cholinergic neurotransmission and synaptic plasticity concerning memory processing. Neurochem Res 1997;22(4):507–15.
[21] Drever BD, Riedel G, Platt B. The cholinergic system and hippocampal plasticity. Behav Brain Res 2011;221:505–14.
[22] Kumar A. Long-term potentiation at CA3–CA1 hippocampal synapses with special emphasis on aging, disease, and stress. Front Aging Neurosci 2011;3(7):1–20.
[23] Li Z, Wu CF, Pei G, Xu NJ. Reversal of morphine-induced memory impairment in mice by withdrawal in Morris water maze possible involvement of cholinergic system. Pharmacol Biochem Behav 2016;8:507–13.
[24] Hozumi S, Nakagawasai O, Tan-No K, Niijima F, Yamadera F, Murata A, et al. Characteristics of changes in cholinergic function and impairment of learning and memory-related behavior induced by olfactory bulbectomy. Behav Brain Res 2003;138:9–15.
[25] Damodaran T, Tan BWL, Liao P, Ramanathan S, Lim GK, Hassan Z. *Clitoria ternatea* L. root extract ameliorated the cognitive and hippocampal long-term potentiation deficits induced by chronic cerebral hypoperfusion in the rat. J Ethnopharmacol 2018;224:381–90.
[26] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd edition San Diego, California, USA: Academic Press; 1986.
[27] Gagolewicz PJ, Dringenberg HC. NR2B-subunit dependent facilitation of long-term potentiation in primary visual cortex following visual discrimination training of adult rats. Eur J Neurosci 2011;34:1222–9.
[28] Easton A, Douchamps V, Eacott M, Lever C. A specific role for septohippocampal acetylcholine in memory? Neuropsychologia 2012;50:3156–68.
[29] Choi BR, Kwon KJ, Park SH, Jeon WK, Han SH, Kim HY, et al. Alternations of septal-hippocampal system in the adult wistar rat with spatial memory impairments induced by chronic cerebral hypoperfusion. Exp Neurobiol 2011;20(2):92–9.
[30] Gottfries CG, Blennow K, Karlsson I, Wallin A. The neurochemistry of vascular dementia. Dementia 1994;5:163–7.
[31] Swartz RH, Sahlas DJ, Black SE. Strategic involvement of cholinergic pathways and executive dysfunction: does location of white matter signal hyperintensities matter? J Stroke Cerebrovasc Dis 2003;12(1):29–36.
[32] Molchan SE, Mellow AM, Lawlor BA, Weingartner HJ, Cohen RM, Cohen MR, et al. TRH attenuates scopolamine-induced memory impairment in humans. Psychopharmacology 1990;100:84–9.
[33] Mandel RJ, Thal LJ. Physostigmine improves water maze performance following nucleus basalis magnocellularis lesions in rats. Psychopharmacology 1988;96(3):421–5.
[34] Zheng XG, Li XW, Yang XY, Sui N. Effects of scopolamine and physostigmine on acquisition of morphine-treated rats in Morris water maze performance. Acta Pharmacol Sin 2002;23(5):477–80.
[35] Xi Y, Wang M, Zhang W, Bai M, Du Y, Zhang Z, et al. Neuronal damage, central cholinergic dysfunction and oxidative damage correlate with cognitive deficits in rats with chronic cerebral hypoperfusion. Neurobiol Learn Mem 2014;109:7–19.
[36] Pappas BA, Torre-de la JC, Davidson CM, Keyes MT, Fortin T. Chronic reduction of cerebral blood flow in the adult rat: late-emerging CA1 cell loss and memory dysfunction. Brain Res 1996;708(1–2):50–8.
[37] Vicente E, Degerone D, Bohn L, Scornavaca F, Pimentel A, Leite MC, et al. Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. Brain Res 2009;1251:204–12.
[38] Blin J, Ivanoiu A, Coppens A, De Volder A, Labar D, Michel C, et al. Cholinergic neurotransmission has different effects on cerebral glucose consumption and blood flow in young normals, aged normals, and Alzheimer's disease patients. Neuroimage 1997;6(4):335–43.
[39] Peruzzi P, von Euw D, Lacombe P. Differentiated cerebrovascular effects of physostigmine and tacrine in cortical areas deafferented from the nucleus basalis magnocellularis suggest involvement of basalocortical projection in microvessels. Ann N Y Acad Sci 2000;903:394–406.
[40] Scremin OU, Scremin AM, Heuser D, Hudgell R, Romero E, Imbimbo BP. Prolonged effects of cholinesterase inhibition with eptastigmine on the cerebral blood flow-metabolism ratio of normal rats. J Cereb Blood Flow Metab 1993;13(4):702–11.
[41] Xu J, Wang Y, Li N, Xu L, Yang H, Yang Z. L-3-n-butylphthalide improves cognitive deficits in rats with chronic cerebral ischemia. Neuropharmacology 2012;62:2424–9.
[42] Bayat M, Sharifi MD, Haghani M, Shabani M. Enriched environment improves synaptic plasticity and cognitive deficiency in chronic cerebral hypoperfused rats. Brain Res Bull 2015;119:34–40.
[43] Markram H, Segal M. Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. J Physiol 1990;427:381–93.
[44] Ovsepian SV, Anwyl R, Rowan MJ. Endogenous acetylcholine lowers the threshold for long-term potentiation induction in the CA1 area through muscarinic receptor activation: in vivo study. Eur J Neurosci 2004;20:1267–75.
[45] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008;14:837–42.
[46] Xu B, Li XX, He GR, Hu JJ, Mu X, Tian S, et al. Luteolin promotes long-term potentiation and improves cognitive functions in chronic cerebral hypoperfused rats. Eur J Pharmacol 2010;627:99–105.
[47] Wei N, Zheng K, Xue R, Ma SL, Ren HY, Huang HF, et al. Suppression of microRNA-9-5p rescues learning and memory in chronic cerebral hypoperfusion rats model. Oncotarget 2017;8(64):107920–31.