Human and Animal Physiology

Recognition Memory Impairment and Neuronal Degeneration Induced by Intracerebroventricular or Intrahippocampal Administration of Okadaic Acid

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ABSTRACT. In the present study, we evaluated and compared effect of intracerebroventricular (ICV) and intrahippocampal bilateral microinjection of okadaic acid (OA) on recognition memory function assessed in open field paradigm and hippocampal piramidal cell loss in rats. Rats were divided in the following groups: Control(icv) - rats injected ICV with aCSF; Control(hipp) - rats injected intrahippocampally with aCSF; OAicv - rats injected ICV with OA; OAhipp - rats injected intrahippocampally with OA. Nissl staining of hippocampal sections showed that the number of pyramidal cells in the CA1 region of the hippocampus in the control group is significantly higher than that in the OAhipp and OAicv groups. The number of pyramidal cells in OAicv group is significantly higher than that in the OAhipp. The results of behavioral study indicate that bilateral microinjection of OA into the dorsal hippocampus induced impairment in recognition memory. Control rats as well as OAicv treated rats clearly reacted to the modification of the configuration by exploring the displaced (or novel) object more than nondisplaced (or familiar) ones. The present findings indicate correlation between recognition memory impairment and hippocampal cell loss induced by OA treatment. Our results give the possibility to assume the involvement of the hippocampus in object and spatial recognition memory and it may be suggested that the OA-induced recognition memory impairment may be attributed, at least in part, to the hippocampal cell death caused by the drug. *© 2015 Bull. Georg. Natl. Acad. Sci.*

Key words: *okadaic Acid, recognition memory, hippocampus, rat.*

Alzheimer's disease (AD) is a neurodegenerative disease that causes progressive cognitive and behavior impairment in the elderly. To date, not only is there no cure for AD, but also the cause and the factors that underlie the progression of AD are not well known. Very few species are known to develop the behavioral, cognitive and neuropathological symptoms of AD spontaneously. The most widely used animal models of AD is transgenic mice. However, transgenic mouse models have some limitations [1].

Unlike the human neuropathology only very few models show neuronal death and most of the commonly used tau transgenic models are associated with the development of motor impairments, which limit the use of these models in behavioral tests [2]. Some pharmacological methods, such as chemical lesions, are used to produce AD models, which are valuable for studying certain pathological pathways [3-5].

It is widely believed that changes in the cerebral activity of protein phosphatases (PP) have been implicated in the pathogenesis of AD. Affected brains of AD are characterized by the presence of senile plaques, neurofibrillary tangles and the loss of cholinergic neurons in the basal forebrain. Neurofibrillary tangles result from accumulation of paired helical filaments within neurons, and such filaments consist largely of hyperphosphorylated tau protein [6]. Hyperphosphorylation of tau has been suggested to be caused by an increase in kinase activity or by a decrease in phosphatase activity within the neurons during the development of AD [7]. Alteration of the normal rates of phosphorylation and dephosphorylation of proteins may affect neuronal functions. In the central nervous system, protein phosphorylation plays a critical role in the molecular mechanisms, through which neurotransmitters and hormones produce their biological effects in target cells [8].

Okadaic acid (OA), a polyether C38 fatty acid toxin extracted from a black sponge *Hallichondria okadaii*, is a potent and selective inhibitor of protein phosphatase, PP1 and protein phosphatase 2A (PP2A). Because of its property to inhibit phosphatase activity, OA is associated with protein phosphorylation and has been proved to be a powerful probe for studying the various regulatory mechanisms and neurotoxicity. Intracerebroventricular (ICV) administration of OA causes neurotoxicity, which is associated with increased intracellular Ca^{2+} level, oxidative stress, and mitochondrial dysfunction in the brain areas [9].

In the present study we evaluated and compared effect of ICV and intrahippocampal bilateral microinjection of OA on recognition memory function and hippocampal piramidal cell loss in rats.

Material and Methods

Subjects. A total of 23 male rats, approximately 4 months of age and weighing 220-250 g at the start of experimentation served as subjects. Rats were divided in following groups: Control(icv) - rats injected ICV with aCSF (n=4); Control(hipp) - rats injected intrahippocampally with aCSF (n=4); OAicv - rats injected ICV with OA (n=8); OAhipp - rats injected intrahippocampally with OA (n=8).

Surgery. Rats were anaesthetized with i.p. injection of 4 % chloral hydrate (9 ml/kg) and placed in a stereotaxic apparatus. OA was dissolved in artificial cerebrospinal fluid (aCSF) and injected ICV (A: 0,2 mm from bregma, L: 1,1mm and V: 3,6mm) 200 ng in a volume of 10 μl bilaterally. Vehicle control received 10 μl of aCSF ICV bilaterally. Into the dorsal hippocampus OA (100 ng in 1 μl saline) or saline (1 μl) was injected bilaterally (A: -4,2 mm from bregma, L: 2.8mm and V: 2,8mm). OA or saline was injected over a 5 min period, and the injection cannula was left in place for an additional 5 min to allow for diffusion of OA away from the injection site. All injections were made with a 1-μl Hamilton syringe with a microinjection pump (**CMA 402 Syringe Pump, Sweden).** The rats were allowed to recover from the surgery for two weeks before starting the behavioral experiments.

Behavioral apparatus. An open-field square arena (65 x 65 x 75 cm) enclosed by walls made from wood and illuminated by a 60 W light bulb mounted 1 m above the area was used for the behavioral test. The floor of the arena was divided into 16 equal squares by white lines. An overhead camera and a video recorder were used to monitor and record the animal's behavior for subsequent analysis.

Behavioral procedure. Rats were individually given five 3-min sessions, each of which was separated by 24-hour delay. During Session 1 four different (by color, shape and size) objects (A, B, C, D) were simultaneously present in the open field. All rats were given

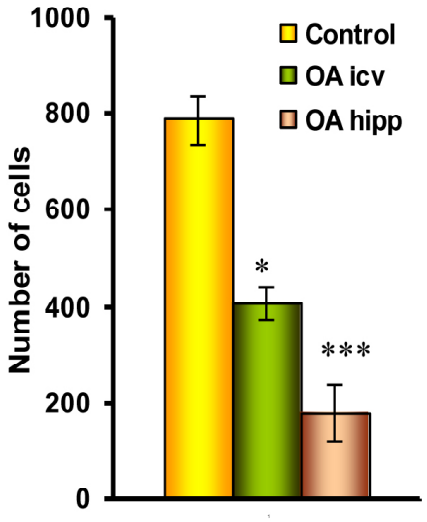

Fig. 1. OA-induced pyramidal cell loss in the CA1 region of hippocampus.
Results are expressed as means ± SEM (n = 7-8 in each group).
***P ≤ 0.001 vs CON and **P ≤ 0.01 vs OA.

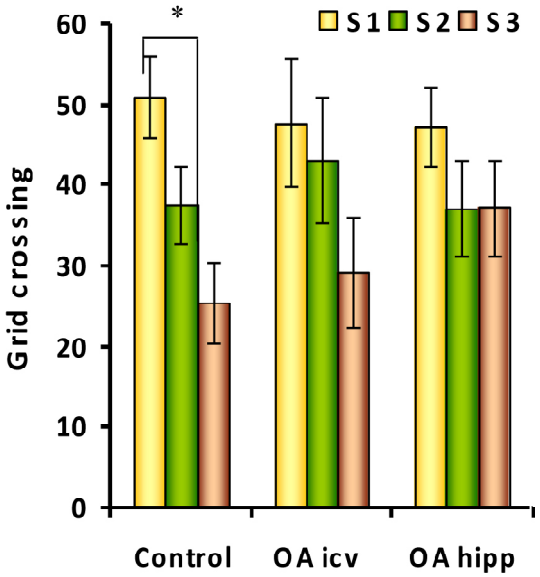

Fig. 2. Effect of OA treatment on the habituation to the environment. The decrease
in the number of crossings between Session 1(S1), Session 2 (S2) and
Session 3 (S3) was taken to be a measure of habituation to the environment.
*Data are given as mean ± SEM. *P ≤ 0.05*

three habituation sessions. For Session 4, the spatial location of the object (B) was modified and in Session 5, one of the familiar nondisplaced object (C) was substituted with a new one (object E) at the same location. The time spent exploring the novel and familiar or displaced and nondisplaced objects were recorded in Sessions 4 and 5 for 3 min.

Behavioral measures. Locomotor activity was assessed by counting the number of grid crossed by each animal while moving in the open field and the amount of time spent by each animal for the object exploration was recorded. The rats' responses to the spatial change (in Session 4) and object novelty (in Session 5) were evaluated as discrimination indexes (DIs) that takes into account individual differences in the total amount of exploration. The following equation was used for displacement discrimination index, DI_D: $DI_D = t_D/(tN_D + t_D)$, where t_D = exploration time of the displaced objects and tN_D = mean exploration time of the non-displaced objects. The object novelty discrimination index, DI_N was calculated as: $DI_N = t_N/(t_F + t_N)$, where t_N = exploration time of the novel object and t_F = mean exploration time of the familiar objects [10].

Histology. At the end of the behavioral experiments OA treated and control rats were deeply anesthetized with pentobarbital and perfused through the ascending aorta with 300 ml saline followed by 600 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The surviving pyramidal cells in the hippocampus of rats were visualized by Nissl staining The number of the hippocampal pyramidal cells in Nissl staining sections was counted at X 400 magnification. Stained sections were analyzed with fluorescence optic microscope Leica MM AF.

Statistical analysis. Statistical analysis were made using ANOVA (SigmaStat statistical software). All data are presented as mean ± standard error of the mean. Differences were considered significant when $p < 0.05$.

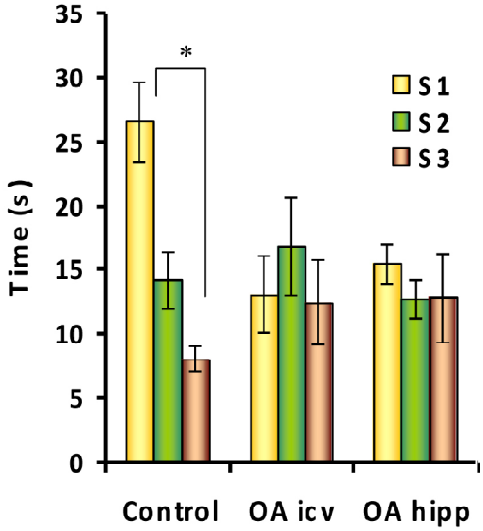

Fig. 3. Effect of OA treatment on the habituation to the objects. The decrease in the time spent for exploring four objects between Session 1 (S1) and Session 3 (S3) was taken to be a measure of habituation to the objects.
*Data are given as mean ±SEM. * $P \leq 0.05$.*

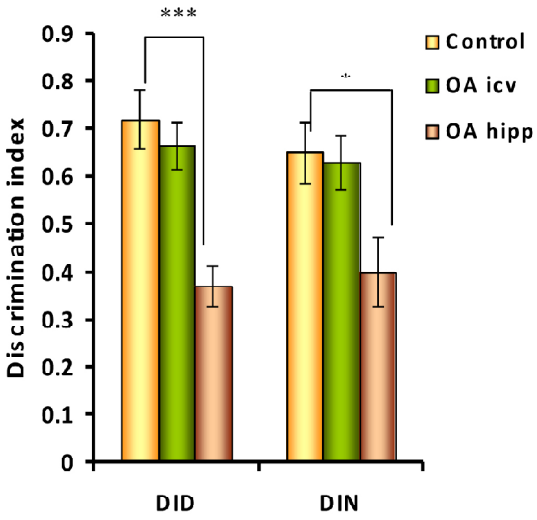

Fig. 4. Effect of OA treatment on the behavioral response to the spatial change (DID) and object change (DIN). The histograms represent difference between spatial change scores or object change scores defined by discrimination indexes (DID - Displacement discrimination index; DIN - The object novelty discrimination index; calculation see in section: methods; behavioral measures).
Data are given as mean ±SEM. * $P \leq 0.05$. *** $P \leq 0.001$.

Results and Discussion

Of the 8 control rats one animal died before the end of the experiment and were excluded from the analysis. Since there were no significant differences (P>0.05) between Control(icv) and Control(hipp) rats with regard to activity, habituation and response to a novelty, these groups were combined into a single one, as of now designated as control (n=7).

Nissl staining of hippocampal sections showed that the number of pyramidal cells in the CA1 region of the hippocampus in the control group is significantly higher than that in the OAhipp (P<0,001) and OAicv groups (P<0,01; Fig. 1). The number of pyramidal cells in OAicv group is significantly higher than that in the OAhipp (P<0,01).

The Two-Way ANOVA for the locomotor activity showed no significant effect of group ($F_{2,68}$=0,142, P=0.868), but showed significant effect of session ($F_{2,68}$=6,357, P=0.003). The interaction between group and session was not significant ($F_{4,68}$=0,603, P=0.662). Post hoc analysis for the locomotor activity showed significant difference (P=0.019) between Sessions 1 and 3 in control group (P=0.019) and no significant difference in OAicv and OAhipp (P=0.085, P=0.475, respectively). The decrease in the number of crossings between Session 1 and Session 3 was taken to be a measure of habituation to the environment. Figure 2 shows effect of OA treatment on the habituation to the environment

The Two-Way ANOVA for the object exploration showed no significant effect of group ($F_{2,68}$=0,763, P=0.471), but showed significant effect of session ($F_{2,68}$=5,290, P=0,008). and significant interaction between group and session ($F_{4,68}$=3,706, P=0.009). Post hoc analysis for the object exploration showed significant difference (P=0.019) between Sessions 1 and 3 in control group (P=0.019) and no significant difference in OA treated groups (OAicv - P=0.985, OAhipp - P=0.766). The decrease in the time spent for exploring four objects between Session 1 and Session 3 was taken to be a measure of habituation to the objects. Figure 3 shows effect OA treatment on the habituation to the objects.

Figure 4 shows the difference between the responses to spatial change (defined by DID in Ses-

sion 4) and to the object novelty (defined by DIN in Session 5) by control and OA treated rats. One-way ANOVA for the displacement discrimination index revealed significant effect of group ($F_{2,22}$=13,243, P<0.001). Post hoc analysis showed a significant difference between the control and OAhipp groups (P<0.001) and no significant difference between the control and OAicv groups (P=0.711). One-way ANOVA for the object novelty discrimination index revealed significant effect of group ($F_{2,22}$=4,558, P=0.023). Post hoc analysis showed a significant difference between the control and OAhipp groups (P=0.038) and no significant difference between the control and OAicv groups (P=0.976).

This experiment compares effect of ICV and intrahippocampal bilateral microinjection of OA on recognition memory function and hippocampal pyramidal cell loss in rats. Nissl staining in the present study showed a marked neuronal destruction of the CA1 region following direct microinjection of OA into the dorsal hippocampus. This is in accordance with previous studies that showed the OA induced hippocampal neurodegeneration [11]. On the other hand, the present study showed that hippocampal cell loss is lower in the OAicv group. Interesting to note that excitotoxic neuronal death associated with neurodegenerative disorders is linked to excessive activity of excitatory neurotransmitters. It is well recognized that blockade glutamatergic NMDA-Rs leads to impairment of neuronal plasticity [12] while their overactivation leads to cell death due to calcium overload [13]. Ekinci et al. [14] found that OA increases Ca^{2+} in hippocampal neuronal cell culture through the ionotropic excitatory amino acid receptors resulting in neuronal degeneration. It is possible to suggest that in our experiments OA induces rise in level of intracellular Ca^{2+} through NMDA receptor that leads to hippocampal cell death.

Hippocampus is the major brain area implicated in learning and memory function. Our data point out that as a result of OA treatment, structural disorganization of the hippocampus is associated with alterations in learning and memory. It is likely that OA-induced memory impairment in the present paradigm is due to the secondary effect of OA-induced hippocampal cell death. The most interesting fact is the certain coincidence of structural and behavioral alterations. Our findings indicate correlation between recognition memory impairment and hippocampal cell loss induced by OA treatment. The results of behavioral study indicate that bilateral microinjection of OA into the dorsal hippocampus induced impairment in recognition memory. Control rats as well as OAicv treated rats clearly reacted to the modification of the configuration by exploring the displaced (or novel) object more than nondisplaced (or familiar) ones. These findings suggest that the OAicv treatment do not disrupt the function of the hippocampus to a sufficient extent to impair recognition memory. It is interesting to note that lesion size is critical factor influencing whether impaired recognition memory is detected after hippocampal damage. Hippocampal lesions are more likely to result in impaired recognition memory when the lesion size is large (>75%) [15]. Indeed, it is not surprising that OAicv induced hippocampal damage does not produce an effect equivalent to that of direct microinjection of OA into the dorsal hippocampus. Bilateral microinjection of OA into the hippocampus, which induced memory impairment, induced a massive lesion of the hippocampal tissue in the OA-injected rats. One important aspect that has to be considered is that the rats of OAicv group failed to habituate to the unchanged environment or objects but they were normal in detecting the spatial or object novelty. It may be suggested that despite decreased habituation, the ability to encode or store a representation of the context in which the objects are encountered are to a certain extent spared, which is sufficient for detection of changes in the environment.

In conclusion, our results give the possibility to assume the involvement of the hippocampus in object and spatial recognition memory and it may be suggested that the OA-induced recognition memory impairment may be attributed, at least in part, to the hippocampal cell death caused by the drug.

ადამიანისა და ცხოველთა ფიზიოლოგია

ტვინის პარკუჭებსა და ჰიპოკამპში ოკადაიკის მჟავის მიკროინექციით განპირობებული ამოცნობის მეხსიერების დარღვევა და ნეიროდეგენერაცია

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წარმოდგენილ ნაშრომში შეისწავლებოდა ოკადაიკის მჟავის (OA) ვირთაგვების ტვინის პარკუჭებსა და ჰიპოკამპში ორმხრივი მიკროინექციის ეფექტები ჰიპოკამპის პირამიდული ნეირონების დაღუპვასა და ამოცნობის მეხსიერებაზე ღია ველის პარადიგმაში. საკონტროლო ჯგუფის ცხოველებში ხდებოდა ტვინის პარკუჭებსა ან ჰიპოკამპში არტიფიციალური ცერებროსპინალური სითხის შეყვანა. ნისლის მეთოდით შეღებილი ტვინის ანათლების კვლევით ექსპერიმენტული ჯგუფის ცხოველებში გამოვლინდა ჰიპოკამპის CA1 ველში პირამიდული ნეირონების სარწმუნო შემცირება საკონტროლო ჯგუფის ცხოველებთან შედარებით. თუმცა, პარკუჭებში OA–ს შეყვანის პირობებში ჰიპოკამპის პირამიდული ნეირონების დაღუპვის ხარისხი ნაკლებად არის გამოხატული. ქცევითი ექსპერიმენტებით გამოვლინდა, რომ აღნიშნული დოზით ოკადაიკის მჟავას მიკროინექცია ჰიპოკამპში იწვევს როგორც ჰაბიტუაციის, ასევე ამოცნობის მეხსიერების გაუარესებას, ხოლო ტვინის პარკუჭებში ოკადაიკის მჟავას მიკროინექციის პირობებში ვლინდება მხოლოდ ჰაბიტუაციის პროცესის გაუარესება. მიღებული შედეგები საფუძველს გვაძლევს ვივარაუდოთ, რომ ჰიპოკამპი ჩართულია ამოცნობის მეხსიერების პროცესებში და OA–ს შეყვანით გამოწვეული ამოცნობის მეხსიერების გაუარესება შესაძლოა უკავშირდება ამ ტოქსინის გავლენით ჰიპოკამპში ნეირონთა დაღუპვას.

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