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Original Article

The Effect of ICV Administration of PI3K on Memory

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Abstract

Introduction: Brain insulin receptors (IRs) have been suggested as an important regulatory factor for cognitive functions but the involvement of IR signaling in memory deficit associated with neurodegenerative conditions is not yet explored. Among the diverse signaling pathways of IR, PI-3 kinase and (MAP) kinase pathways in brain have been suggested for learning and memory functions. The phosphoinositide3-kinase (PI3K) complex plays important roles in virtually all cells of the body. The enzymatic activity of PI3Kto phosphorylate phosphoinositides in the membrane is mediated by a group of catalytic and regulatory subunits. Among those, the class I catalytic subunits, p110α, p110β,p110γ,and p110δ have recently drawn attention in the neuroscience field due to their specific dysregulation in diverse brain disorders. The present study was planned to investigate the effect of PI3K on memory.

Materials and Methods: The animals were injected bilaterally with ICV water (control group), ICV PI3k (1,10 and 100 ng/rat) on days 1 and 3 after surgery. The learning and memory performance was assessed two weeks after the first dose of drugs by using step-through passive avoidance paradigm (0.3 mA, 3seconds) and open field test. The results revealed that The ICV administration of PI3K (P<0.05) altered inhibitory avoidance acquisition. PI3K at dose 1 ng/rat decreased the step- through latency during the retention test.

Results: data showed that PI3K at dose of 1 ng/rat decreased the step- through latency during the retention test. In addition, the results showed that PI3K at dose 10 ng/rat increased locomotor activity.

Conclusion: Finally, our data indicated that PI3K has critical role in memory consolidation and locomotor activity.

Keywords: PI3K, Passive Avoidance, Memory, Rat

1. Introduction

The presence of brain insulin receptors (IRs) in the hippocampus suggests its functional involvement in cognition [1]. Hippocampal IR system might play an important role in the regulation of memory functions [2, 3]. Moreover, deregulation of brain IR has been linked to the pathogenesis of age-related neurodegenerative disorders such as Alzheimer's [4] and Parkinson's disease [5].

The molecular cascades downstream from IR are composed of a large number of signaling molecules including insulin receptor substrates (IRSs) and Src homology 2 (SH2) and SH2-SH3 domain-containing proteins. Among the diverse signaling pathways of IR, insulin receptor substrate-1 (IRS- 1)/PI-3 kinase/phosphoinositide-dependent kinase (PDK)/ protein kinase B (PKB/Akt) and the Src homology and collagen protein

(Shc)/growth factor receptor-bound protein-2 (Grb2)/mitogen-activated protein (MAP) kinase pathways in brain have been suggested for learning and memory functions [6].

Studies showed that the formation of long-term extinction memory is associated with activation of kinase cascades, transcription factors, and proteins [7-9]. The phosphoinositide 3-kinase (PI3K) has been found vital for cell proliferation and prevention of apoptosis. A well-documented PI3K downstream target is AKT/ protein serine/threonine kinase (PKB); the activation of AKT is usually dependent on PI3K, and its phosphorylation is blocked by PI3K inhibitors. Increasing evidence indicates that PI3K has a role in mediating the formation of memory [9-11].

Further, extracellular signal-regulated kinase (ERK) can be activated by other signaling cascades that are critical for Hippocampal-dependent memory formation, including phosphatidylinositol 3-kinase/Akt (PI3K/Akt) [12]. PI3K is crucial for hippocampal plasticity and object recognition [10, 13, 14], is vital for neuroprotection [15], which could be critically important for the aging brain.

Signaling through phosphoinositide 3-kinases (PI3Ks) has diverse roles in the human body, regulating essential functions such as cell growth, migration, differentiation and survival. PI3K signaling is important for an adequate immune response [16], hematopoiesis [17], and organ growth [18]. Mutations in PI3K catalytic subunits were found in primary immune deficiencies [19] and in different forms of human cancer, including leukemia [20, 21]. Apart from a role in dividing cells, PI3K activity is also a key regulator of neuronal function. PI3K signaling transduces signals from cell surface receptors to the Akt/ mammalian target of rapamycin (mTOR) pathway and is essential for synapse and dendritic spine development [22-25] and for enduring forms of synaptic plasticity underlying learning and memory [26-30]. Therefore, it is not surprising that an increasing body of evidence suggests dysregulated PI3K activity and down-stream signaling as a key contributor and potential therapeutic target for mental disorders [31-35].

In vertebrates, PI3K enzymatic activity is brought about by eight different catalytic subunits. These catalytic subunits are divided into class I, class II, and class III. PI3K enzymes according to their protein structure, function and associate with regulatory subunits [36]. Most recently, p110 subunit-specific functions and mechanisms have begun to be discovered in the brain. The different p110 isoforms appear to have unique roles in mediating distinct forms of neuronal function and synaptic plasticity, suggesting the use of subunit-selective p110 inhibitors for certain brain disorders. The importance of PI3K catalytic subunit-selective roles in neurons is illustrated by functional and genetic studies that have linked dysregulation or mutations of specific p110 isoforms with distinct brain disorders. Given the essential function of PI3K signaling in non-neuronal cells, a precise knowledge of the molecular mechanisms of neuron-specific PI3K enzyme regulation and dysregulation in disease is mandatory for the development of therapeutic strategies ameliorating brain disorders without compromising other essential functions of the body [37].

Considering the involvement of IR and its signaling pathways in the hippocampus,The present study was planned to investigate neuroprotective effects of PI3K on memory formation. For this purpose,we examined the effect of ICV administration of PI3K in acquisition of inhibitory avoidance memory.

2. Materials and Methods

2.1. Animals

Adult Male Wistar rats, weighting 200-220 g were purchased from Pasteur

Institute, Tehran, Iran. There were 8 rats in each group. Animals were housed in standard cages under controlled room temperature (22–24 °C), humidity (45–50%) and light exposure conditions 12:12 h light–dark cycle (light on at 08:00 am). All experiments were carried out during the light phase of the cycle (8:00 am–3:00 pm). The animals had unrestricted access to food and water. Animals were allowed to adapt to the laboratory conditions for at least 1 week before surgery. All experiments were performed between 9:00 and 15:00 and each rat was tested only once. All procedures in this study are in accordance with the guide for Care and Use of Laboratory Animals as adopted by the Ethics Committee of Faculty of Science, Tehran University (357:November 2000).

2.2. Drugs

The following drug was used in the experiments: PI3K (Sigma-Aldrich Company, USA, stored at −80 °C).
The drug was administered intracerebroventricularly (ICV) twice at an interval of 48 on days 1 and 3 after cannulation. PI3K at dose of 1,10 and 100 ng/rat dissolved in 2 µl sterile deionized/distilled water and 1µl injected slowly by micro liter syringe (Hamilton) on each side using the coordinates.
The infusion process lasted 2 to 3 minutes. One minute after infusion, the needle was removed very slowly from the site. The infusion process lasted 2 to 3 minutes. One minute after infusion, the needle was removed very slowly from the site.

2.3. Intracerebroventricular (ICV) Injection

The rats were anesthetized by i.p. infusion of Ketamine (60mg/kg) and Xylazine (6 mg/kg) mixture, and their heads were placed in stereotax apparatus. Located in a site (AP= - 0.8 mm; ML= ±1.4 mm; DV= 3.6 mm) based on the rat brain atlas, each guide cannulae was positioned with a 0.5 mm distance from the lateral ventricles level. The cannulae were secured to the bone with dental acrylic crement.

2.4. Passive Avoidance Task

Passive avoidance memory test performance was examined on the 14th day after 1st day of the injection. The apparatus used for the passive avoidance task was the two-way shuttle box (BorjSanaatAzma Co., Tehran, Iran), which consisted of an illumination compartment with identical dimensions (30 cm × 20 cm × 20 cm) made of transparent plastic and of a dark compartment (30 cm × 20 cm × 20 cm), the walls and ceiling which were made of dark opaque plastic. A rectangular opening (8 cm × 8 cm) was located between the two chambers and could be closed by an opaque guillotine door. The grid floors of both chambers were made of stainless steel rods (2mm diameter), spaced 1 cm apart. The apparatus was kept in an acoustically insulated room, under standard conditions. The experiment consisted of adaptation, training and memory retention sessions. In the adaptation session, rats were put into the illuminated compartment and allowed to enter the dark compartment. After the rats entered the dark compartment, the door was closed and the rats were returned to their home cages. After 30 min of adaptation, the rats were placed in the illuminated compartment for the training session. After the rats entered the dark compartment, the door was closed and an electric shock (0.3 mA, 50 Hz 3 seconds) was delivered from the steel-rod floor. Rats were allowed to explore the light compartment for 20 sec before the door to the dark compartment was opened. The latency to step into the dark compartment was recorded (pre-shock latency). After 20 sec, the rats were returned to their home cages. Two minutes later, rats were placed in the illuminated compartment for 20 sec and after opening the door, the latency of stepping into the dark compartment was recorded. If latency was greater than 120 sec (post-shock latency),

the rats were returned to their home cages and acquisition trial was performed. If latency was less than 120 sec, the shock would be repeated. Memory retention was checked 24 h later. The rats were placed in the illuminated compartment for 20 sec and after opening the door, the latency of stepping into the dark compartment was recorded (test latency) up to a maximum of 5 min. No shock was delivered during the test session.

2.5. Open Field Test

Immediately after the inhibitory avoidance test session, the animals were transferred to an open-field test. The open field apparatus was constructed of white plywood and measured 72 × 72 cm with walls 36 cm high. One of the walls was clear Plexiglas, so rat could be visible in the 2 apparatus. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 × 18 cm squares. A central square (18 cm × 18 cm) was drawn in the middle of the open field [38]. The central square is used because some rat strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Also, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations [39]. The open field session lasted for 5 min and during this time, an observer, who was not aware of the pharmacological treatments, recorded the number of crossing responses and rearing responses manually. This test was carried out to identify motor disabilities, which might influence inhibitory avoidance performance at testing [40].

2.6. Statistical Analysis

Since data displayed normality of distribution and homogeneity of variance, the results were statistically evaluated by analysis of variance one-way (ANOVA). Results were expressed as mean ±S.E.M. and the statistical analysis was done through one-way analysis of variance (ANOVA), followed by the Newman–Keuls (post –hoc analysis) test to determine the significance of difference among various groups. The p-value< 0.05 was considered as statistically significant

3. Results

One-way ANOVA analysis revealed that ICV administration of PI3K [F (3, 28) =5.1681, P<0.05, Fig.1A], altered inhibitory avoidance acquisition. Moreover, post hoc analysis showed that PI3K at a dose of 1 ng/rat decreased the step-through latency during the retention test and the other doses had no effect on memory formation using the passive avoidance task.

In addition, one-way ANOVA showed that PI3K [F (3, 28) =16.190, P<0.01, Fig.1B], at a dose of 10 ng/rat increased locomotor activity and the other doses had no effect on locomotor activity using the open field test.

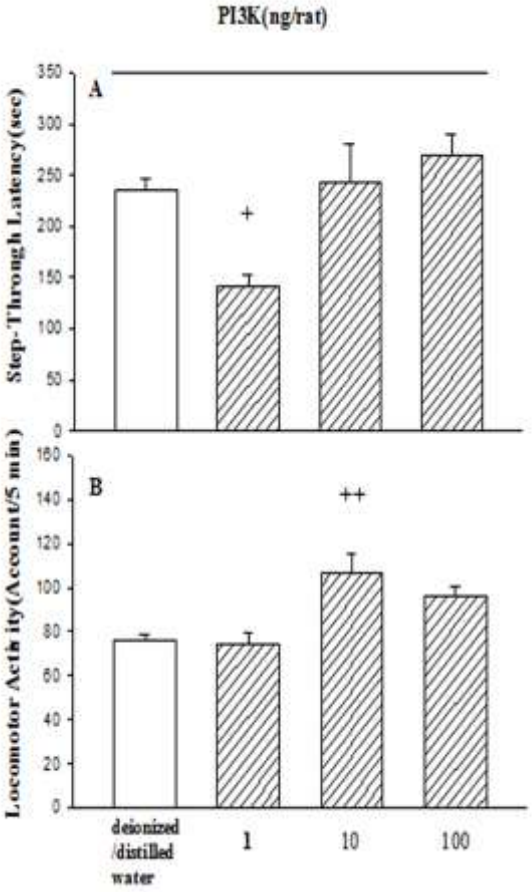

Figure 1. The effect of PI3K on memory and locomotor activity. The animals received ICV PI3 K or sterile deionized/distilled water according to the table. All drugs were injected on day 1 and 3 after surgery. The memory was measured two weeks after first dose ICV injection. B showed the effect of drugs on locomotor activity in test day. Data are expressed as mean ±S.E.M of eight animals per group. *+P<0.05* and *++P<0.01* different from sterile deionized/distilled water/PI3K control group

4. Discussion

In our study, data revealed that ICV administration of PI3K altered inhibitory avoidance acquisition. Moreover, the analysis showed that PI3K at a dose of 1 ng/rat decreased the step-through latency during the retention test. In addition, the results showed that PI3K, at a dose of 10 ng increased locomotor activity.

This is the first time that ICV administration of PI3K is examined in memory experiments. Extensive evidence showed that AKT is the main kinase of the phosphoinositide 3-kinase cascade (PI3K) and has been implicated in long-term memory but in these experiments, all researchers used the inhibitors of AKT or ERK kinase pathways.

The other findings provide additional evidence suggesting that targeting PI3K has differential effects depending on the temporal and spatial parameters of time and site of injection [11, 41]. The presumed involvement of AKT in synaptic plasticity and memory processes suggests that its phosphorylation levels should be changed when learning occurs. The results show different activation of AKT following conditioned taste aversion (CTA) conditioning and extinction. Surprisingly, although AKT is a downstream target of PI3K which undergoes dephosphorylation following extinction, its inhibition is not obligatory for CTA acquisition or extinction [42]. Alterations in IR expression in membrane fraction of CA1 and CA3 may be

linked with learning and memory (6). A significant increase in IR/IRS-1/Akt pathway, including IRS-1 and Akt phosphorylation was observed after water maze training in membrane in these regions. Increase in Akt phosphorylation consistent with the up-regulation of Akt gene expression in spatial learning has been reported [43].

The observations point out that IR signaling pathways are associated with learning and memory and the brain IRs are more susceptible to oxidative stress than to cholinergic activity [44].

Some results showed that while AKT phosphorylation was increased following CTA learning, it was decreased following CTA extinction. Inhibition of AKT phosphorylation in the IC before or after the first CTA retrieval test resulted in reduction of the aversion index as re-application of the unconditioned stimulus (lithium chloride) and did not induce the recovery of aversion in LY294002-treated animals. These data also added new evidence to suggest that PI3K was engaged in consolidation of aversive memories, as its inhibition was associated with erasure of CTA memory [45]. The other findings confirmed that PI3K was not involved in the retrieval of CTA memory [11, 12].

Therefore, the result also showed different activation of AKT following CTA conditioning and extinction. Specifically, in the IC, the levels of p-AKT were dramatically increased after CTA conditioning, compared with the control group and extinction training was associated with dephosphorylation of AKT following the extinction session [9].

It has been shown that Protein Kinase A (PKA) was activated by cyclic-AMP. Inhibition of PKA at the time of retrieval of CTA in the BLA resulted in rapid extinction [46, 47]. Another data added new evidence to suggest that PI3K was engaged in consolidation of aversive memories, as its inhibition in the IC led to erasure of CTA memory [48-50].

5. Conclusion

ICV administration of PI3K in some doses had effect on memory consolidation, altered inhibitory avoidance acquisition and locomotor activity. PI3K at a dose of 1 ng/rat decreased the step-through latency during the retention test. In addition, the PI3K, at dose 10 ng increased locomotor activity. Moreover, data showed that PI3K at dose of 1 ng/rat decreased the step-through latency during the retention test. In addition, the results showed that PI3K at dose 10 ng/rat increased locomotor activity.

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Conflict ofinterest

The authors declare no conflict of interest.

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