Effect of antisense oligonucleotide of noggin on spatial learning and memory of rats

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ABSTRACT

AIM: To investigate the effect of antisense oligonucleotide (ASODN) of noggin on rat spatial learning and memory. **METHODS:** Expression of noggin mRNA was measured by *in situ* hybridization method and the ability to spatial learning and memory was tested with Morris water maze. **RESULTS:** Compared with control rats, noggin mRNA positive neurons in dentate gyrus (DG) and CA3 region of hippocampus were markedly increased after the Morris water maze training ($P<0.01$). The increase of noggin mRNA positive neurons in hippocampus following maze training could be significantly blocked by icv injection of antisense noggin ODN, and the injection also impaired the learning and memory formation as compared to that in control rats. But the sense oligonucleotide (SODN) had no effect. **CONCLUSION:** Noggin, as an embryonic gene expressed in adult hippocampus, plays an important role in the process of learning and memory formation.

INTRODUCTION

The gene noggin, originally cloned in Xenopus in 1992, encodes a secreted factor expressed in the Spemann organizer. Its function is to oppose the ventralizing influence of bone morphogenetic proteins (BMP)[1]. Recently, noggin expression has been demonstrated expressed in particular region in adult brain. It is suggested that as one of the earliest acting neural inducers, noggin may be have important roles in the adult nervous system[2]. Furthermore, Ruat and coworkers demonstrated that BMP and their receptors were expressed in adult hippocampus[3]. Noggin as antagonist to BMP acts by binding directly, thereby preventing them from interacting with their receptors[4]. BMP and relative factors including receptors and antagonists may influence brain plasticity which is important for learning and memory[5]. By now, the causal relation between noggin expression in adult hippocampus and memory formation is not clear. In the present study, we investigated the expression of noggin in adult hippocampus and causal relation between noggin expression and memory formation.

MATERIALS AND METHODS

Rats Wistar rats (n=36, weighing180-200 g, Grade II, Certificate No 24301050) of either sex were provided by the Experimental Animal Center of Third Military Medical University. Rats were divided into six groups randomly as following: (1) control group (n=6): rats were injected with 0.9 % saline (10 μL, icv). (2) antisense group (n=6). Animals were given antisense oligonucleotide (ASODN) (icv, 20 μg/d) for 4 d. (3)

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sense group (n=6). Animals were given sense oligonucleotide (SODN) (icv, 20 μg/d) for 4 d. (4) Morris water maze (MWM) group (n=6). Animals were trained 6 trials per day last for 6 d in Morris water maze. (5) MWM+antisense group (n=6). (6) MWM+sense group (n=6).

Drugs and reagents Phosphothioate oligonucleotides were synthesized at Sangon Biological Engineering Technology (Shanghai, China) and dissolved in sterile pyrogen-free 0.9 % saline. The sequences of antisense and sense oligodnucleotide were 5'-TCCAGCTCCGCCAGGTC-3' and 5'-GACCTGGCGGAGCTGGA-3', respectively, which complementary to the transcriptional start site of the rat noggin.

Icv injection The method of antisense and sense oligonucleotides icv microinjection was performed as reference report[6]. Rats were anesthetized with pentobarbital (30 mg/kg, ip) and placed in a stereotaxic apparatus. The indwelling stainless steel cannula was implanted into the right ventricle (anterior -0.3, lateral 1.2, ventral 4.5), according to the atlas of Paxinos and Watson[7]. The rats were allowed a 3-d recovery period after the surgery for implantation of the cannula. The noggin antisense and sense oligonucleotides were administered icv (20 μg oligonucleotides per day) with a microinjector in an amount of 10 μL at a constant rate within 1 min.

Behavioral test with Morris water maze The rats were trained to locate a hidden escaped platform in the circular water pool. The platform always resided in the west quadrant and was submerged 1 cm below the water. After continuous microinjection of oligonucleotide for 4 d, the rats were subjected to Morris water maze training. The rat was placed into the pool, facing the wall of tank and allowed 120 s to locate and climb onto the submerged platform. The rat was allowed to stay on the platform for 30 s, before next training trial, if it failed to find the platform within 120 s, it was guided gently onto platform. Mean latency to find the hidden platform on the water maze task was recorded on each day of testing. The mean performance of a group of rats was measured by 6 trials per day last for 6 d and a 10-min interval between trials for rest. After 14-d delay, rats were tested for location retention of the hidden platform for 4 d.

Tissue preparation One hour after rats finished the last training, they were anesthetized with pentobarbital and intracardially perfused with saline, followed by 300 mL fixative of 4 % paraformaldehyde in phosphate buffer PB (0.1 mol/L, pH 7.4). Following the perfusion, brains were removed and postfixed in the same fixative overnight, then immersed in 30 % sucrose solutions containing 4 % paraformaldehyde in PB 0.1 mol/L until sinking. Serial sections (35 μm) of hippocampus were cut on a freezing microtome and processed for *in situ* hybridization histochemistry reaction.

***In situ* hybridization** The hybridization procedure was according to the method of Bloch *et al*[8]. The probe was digitoxin-labeled antisense cRNA (0.5 μL/mL). And the concentration of anti-digoxigenin antibody was 1:1000. The sections were stained with NBT (4-nitro blue tetrazolium chloride) and BCIP (5-bro-4-chloro-3-indolylphosphate) for 12 h at 4 °C in darkness. No signals were detected in control sections. At present study, neurons which had grain density at least 5 times higher than the background density were considered to show positive expression.

Statistical methods Estimation of noggin mRNA positive neurons was performed on serial hippocampus sections, 10 sections were selected randomly from every group and objectively assessed with an image analyzer. Mean escape latency and the number of noggin mRNA positive neurons were expressed as mean±SD. The differences between any two groups were analyzed by ANOVA, and the differences between two sides within a group by paired t test.

RESULTS

Effect of Morris water maze training on noggin mRNA expression in rat hippocampus By *in situ* hybridization, there were extensively noggin mRNA positive neurons in hippocampus DG and CA3 region in the control group. Rats were continuously given ASODN (20 μg/d, icv) for 4 d, the number of noggin mRNA neurons in DG and CA3 region was decreased by 56.6 % (P<0.01) and 70.0 % (P<0.01) respectively, compared with control group, although SODN had no effect. After maze training for 6 d, densely and deeply stained noggin mRNA positive neurons could be seen in above-mentioned regions, and the greatest number of noggin mRNA neurons was in DG (68±4). In rats continuously given ASODN (20 μg, icv) for 4 d before maze training, the number of noggin mRNA neurons in DG and CA3 region was decreased by 44.8 % and 34.9 % respectively compared with MWM group (Fig 1), whereas the number of noggin mRNA positive neurons had no change in rats pretreated with SODN (Tab 1).

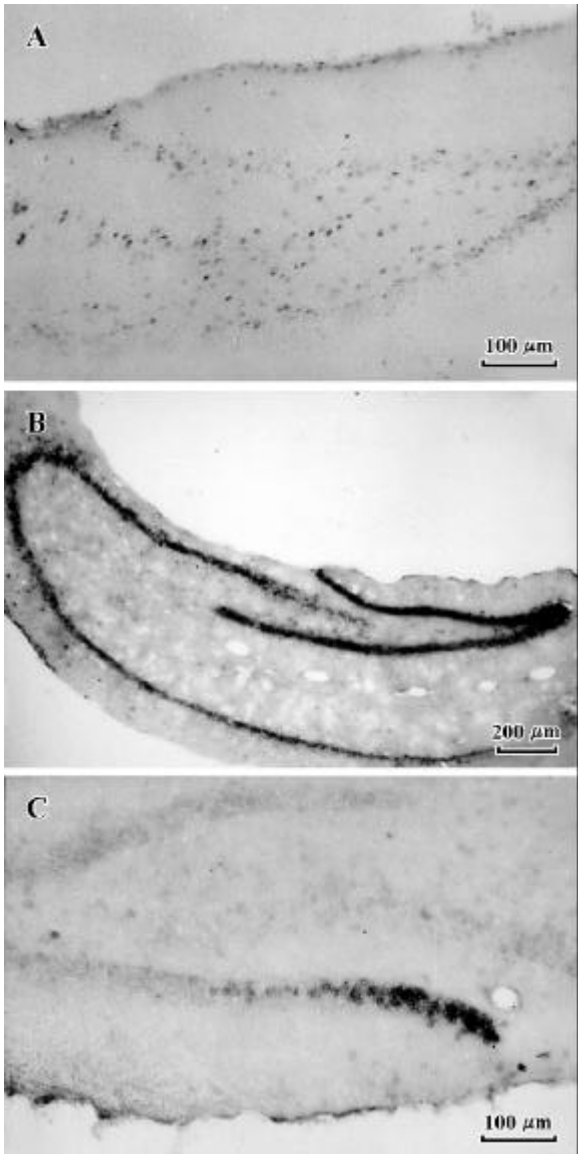

Fig 1. Photomicrographs of noggin mRNA positive neurons in the hippocampus. A) Control group (NBT/BCIP stain, ×100); B) Morris water maze training group (NBT/BCIP stain, ×40); C) Icv ASODN combined with the Morris water maze training group (NBT/BCIP stain, ×100).

Effect of ASODN on spatial learning and memory Rats in control group and SODN group showed a progressively reduced escape latency to find the platform in the Morris water maze during training, while the escape latency of group ASODN was significantly longer than that of control group in each training block during six consecutive days (Fig 2, $P<0.01$). After a 2-week retention period, all rats were retested on this same hidden platform location, the mean latency in rats given ASODN was increased on d 19, and then kept at the same level as to d 6 (Fig 2).

Tab 1. Effect of Morris water maze (MWM) training on noggin mRNA expression in rat hippocampus. *n*=6. Mean±SD. $^cP<0.01$ *vs* control group. $^fP<0.01$ *vs* MWM group.

Group	Number of noggin mRNA positive neurons	
	Dentate gyrus	CA3 region
Control	25.2±1.7	10±3
ASODN	11.2±1.1^c	5.2±1.0^c
SODN	28±3	9.5±2.1
MWM	68±4^c	19.5±2.1^c
MWM+ASODN	19±4^f	5.2±2.0^f
MWM+SODN	60.7±2.5	28.2±2.3

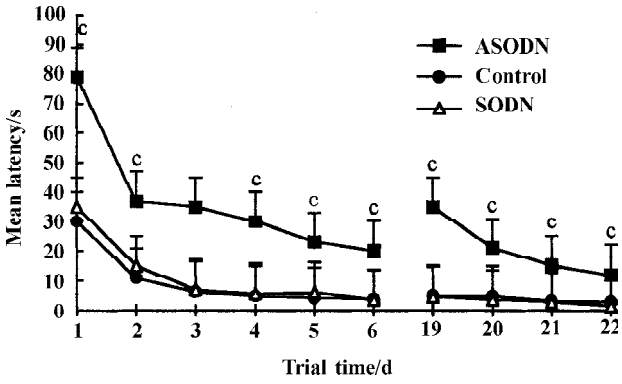

Fig 2. Effect of ASODN on spatial learning and memory. Each point represents the mean latency of rats in each group on the six trials per day. Days 1-6=performance during the acquisition of the initial location of the hidden platform; d 19=performance on testing for retention of the initial platform location after a 14-d delay. *n*=6. Mean±SD. $^cP<0.01$ *vs* control.

DISCUSSION

Hippocampus plays a central role in many memory formation processes, including spatial learning – locating objects in the environment – and consciously recalling facts, episodes, and unique events. Using *in situ* hybridization, we observed that extensive noggin mRNA positive neurons could be seen in hippocampus, and most notably expressed in dentate gyrus (DG) and CA3 region. Furthermore, we demonstrated that the expression of noggin mRNA expression in adult hippocampus could be increased significantly following Morris water maze training hidden platform. These results suggest that noggin mRNA expression in hippocampus may be relative to memory formation.

ASODN, complementary to a select region DNA

messenge RNA (mRNA) encoding the protein of interest, can potentially interfere with transcription and translation thereby decreasing gene expression[9]. In vertebrates, the use of ASODN to unravel molecular bases of behavior is well established. Concerning research on learning and memory, a variety of proteins has been investigated in several vertebrate species using ASODN[10,11]. In our study, we observed that icv injection of noggin ASODN decreased noggin mRNA expression in rats hippocampus significantly. This showed that noggin ASODN could inhibit noggin mRNA expression effectively.

The Morris water maze is a standardized behavioural task to test spatial navigation in rodents. We found that icv administration ASODN not only blocked the enhanced expression of noggin mRNA relevant to the behavioral trainings, but also impaired the memory formation following behavioral trainings. This indicated that noggin expression in adult hippocampus was beneficial to spatial learning and memory.

The mechanism of noggin affecting spatial learning and memory is not clear. But recent research has indicated that this is relative to noggin inducing adult neurogenesis. Adult neurogenesis has been proved to promote the learning and memory[12,13]. Lim *et al* has proved that noggin and bone morphogenetic protein 4 (BMP4) mediate neurogenesis in subventricular zone (SVZ), of which noggin is a positive signal for adult neurogenesis[14]. Noggin and BMP expression in adult hippocampus provide morphological basis for noggin modulating adult neurogenesis in hippocampus. A further study is needed for the better understanding of the mechanism.

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