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The effect of hepatocyte growth factor on learning and memory abilities in a rat model of kainate-induced epilepsy

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ABSTRACT

Background and Objective: Epilepsy as a chronic neurological disorder causes inherent seizures and learning and memory failure. Since there is no acceptable control of seizures in some patients with the current recommended drug therapy, new medications with different mechanisms of action are needed. Here, the beneficial effect of hepatocyte growth factor (HGF) was evaluated in an experimental model of temporal lobe epilepsy in male rats.

Materials and Methods: In the present study, effects of intracerebroventricular administration of HGF (6 µg) thirty minutes before intrahippocampal injection of kainic acid (4 µg) on spontaneous seizures and learning and memory impairment were assessed in rats. As positive control group, valproic acid (200 mg/kg) was injected intraperitoneally.

Results: Behavior data showed that the kainate rats exhibited spontaneous seizures, lower spontaneous alternation score in Y-maze task ($p<0.001$), and impaired learning capability in the passive avoidance test ($p<0.001$). Administration of HGF to kainate rats decreased the numbers of spontaneous seizures, improved alternation score ($p<0.001$) and retention and recall capability in the passive avoidance test ($p<0.001$).

Conclusion: This study revealed that HGF administration to kainate-injected rats attenuates seizure scores and improves learning and memory.

1. Introduction

Epilepsy is a chronic neurological disease with frequent seizures. Clinical local epilepsy complications due to the source of discharge area include sensory, motor, autonomic and psychological symptoms that are associated with relative preservation or loss of consciousness (1, 2). The most frequent localized epilepsy in adults is the temporal lobe epilepsy (TLE) that treatment with conventional drugs has no considerable effect on it (3, 4). The occurrence of epileptic seizures reduces the learning and memory abilities in patients with TLE. Despite significant progress in epilepsy treatment in about one-third of patients, they still do not respond to drugs efficiently and long-term use of these drugs actually do not prevent disease progression.

Different theories have been proposed for etiology of epilepsy (5-8). Among these, changes in the synaptic plasticity and dysfunction of inhibitory (such as GABA) and excitatory (such as glutamate) neurotransmitters (9) play a major role in development of TLE. For this reason, new treatments for improvement of the epilepsy is considered to some extent on the basis of their interference with the function of the neurotransmitters (such as GABA agonists).

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One of the signaling pathways of hepatocyte growth factor (HGF) (10) and its specific receptor (c-MET) (11, 12) is GABAergic inhibitory pathway. Although the effects of neurotrophins on epilepsy are uncertain, but in a previous study on the effect of HGF on epilepsy, it was revealed that mutations in one of the HGF signaling pathways leads to a 50% reduction in GABAergic inhibition (13, 14). In addition, HGF is able to maintain the GABAergic interneurons and improve PTZ-induced seizures (15). In a study on the Wistar rats, it has been shown that injection of the HGF a week before induction of seizures by kainic acid could significantly improve the rats condition based on the score of Racine as well as their electroencephalogram (16).

In this study, the beneficial effects of HGF on learning and memory tasks and seizure scores in a rat model of kainate-induced epilepsy were evaluated using behavioral methods.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (n = 40) (Local animal house), weighing 200-250 g, were maintained three to four per cage in a temperature and natural light-controlled colony room. Animals were given free access to tap water and standard rat chow. Procedures involving animals were made to minimize animal suffering.

2.2. Experimental procedure

Rats were randomly divided into the following groups (n=10): Sham-operated (SH); kainate; HGF (6 µg)-treated kainate and valproate-treated. For stereotaxic surgery, rats were anesthetized with a combination of ketamin (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), placed in a Stoelting stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution and incised on the midline, and a burr hole was drilled through the skull. Animals in the kainate group were unilaterally injected in the dorsal hippocampus with 10 µl of normal saline containing 0.4 µg/µl kainic acid (Sigma Chemicals, USA). HGF (Sigma Chemicals, USA) was dissolved in 30% Cremophor and administered (6µg; i.c.v) 30 minutes before kainic acid injection. The progression of kainate-induced seizures was no reaction 1; stereotype mounting, eye blinking, and/ or mild facial clonus 2; head nodding and/or several facial clonus 3; myoclonic jerks in the forelimbs, clonic convulsions in the forelimbs with rearing 4; and generalized clonic convulsions associated with loss of balance 5 (17).

2.3. Y-maze task

In this study, the recording of spontaneous alternation behavior two weeks post-surgery in a single-session Y-maze was used for assessment of spatial memory (18). Y-maze with three arms was made of black Plexiglas. After putting each unexperienced rat at the end of one arm, it was permitted to move freely into the maze for an 8-min session. The maximum number of possible spontaneous alternations was determined as the total number of arms entered - 2, and the percentage was calculated as the ratio of actual to possible alternations × 100

2.4. Single-trial passive avoidance test

Two days after Y-maze, single-trial passive avoidance test was done according to a previous study (19). This test was performed by the apparatus consisted of a light chamber connected to a dark chamber by a guillotine door. After a habituation period, the guillotine door was lifted, after the rat entering into the dark chamber, the guillotine door was closed and a single electric shock (1 mA, 1 s) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded. After 24 hours, each rat was placed in the light chamber for retention trial. The interval between placement in the light chamber and entry into the dark chamber was measured as step-through latency (STL).

2.5. Statistical analysis

All data were presented as means ± SEM and were analyzed by parametric one way ANOVA followed by Tukey *post hoc* test. Data was regarded significant when p value was less than 0.05.

3. Results

3.1. Behavioral study

Using Racine's classification, it was revealed that 24 hours after intrahippocampal kainic acid injection (the acute period), 5 and 4 seizure scores were observed in 40% and 60% of rats, respectively. After intracerebroventricular administration of HGF, the seizure scores significantly attenuated, so that none of the rats displayed 5 seizure score and 4 seizure score. About 50% of rats showed no Racine's signs (Table 1).

Table 1. Numbers and rates of spontaneous seizures in each group

Group	Class 4 seizure number	Class 5 seizure number	Class 4 seizure Rate%	Class 5 seizure Rate%
Sham	0	0	0	0
Kainate	6	4	60%	40%
Kainate+HGF	2	0	20%	0
Kainate+Valproate	0	0	0	0

3.2. Spatial recognition memory in Y-maze

For assessment of the short-term spatial recognition memory, we recorded the alternation behavior in Y-maze task. In the kainic acid-injected rats, the alternation score significantly decreased comparing to the sham-operated group ($p<0.001$). Treatment of kainate group with HGF at a dose of 6µg increased alternation score in comparison with kainic acid-injected rats ($p<0.001$). After comparing total number of arms entered as an index of locomotor activity, it appeared that kainic acid, HGF and both of them have no significant effect on the total number of entered arms (Fig. 1). There was no significant difference in alternation score between kainate+HGF and kainate+valproate groups.

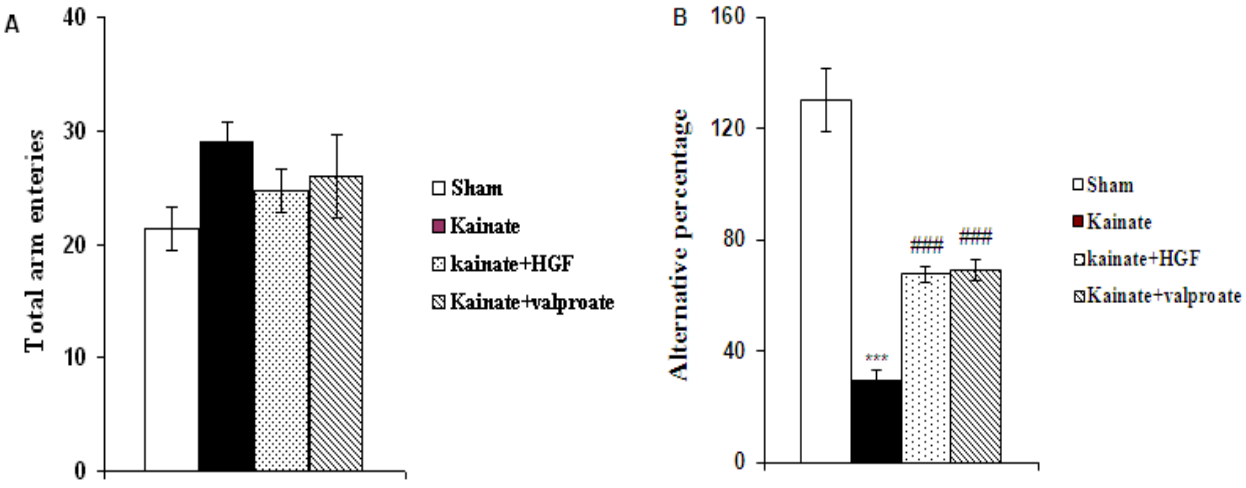

Fig. 1. Total entrance (A) and alternation behavior (B) displayed in the Y-maze by rats. Values are means ± SEM.
*** $p< 0.001$ (vs. Sham); ### $p < 0.001$ (vs. Kainate)

3.3. Passive avoidance test

As shown in Fig. 2, comparing initial latency and step through latency in different groups revealed that regarding IL, there was no considerable difference between experimental groups. But it became evident that kainic acid injection significantly decreases STL as compared to sham group ($p<0.001$). Intracerebroventricular injection of HGF caused the STL to significantly improve in kainate-injected rats ($p<0.001$). No considerable difference was observed in STL between kainate+HGF and kainate+valproate groups.

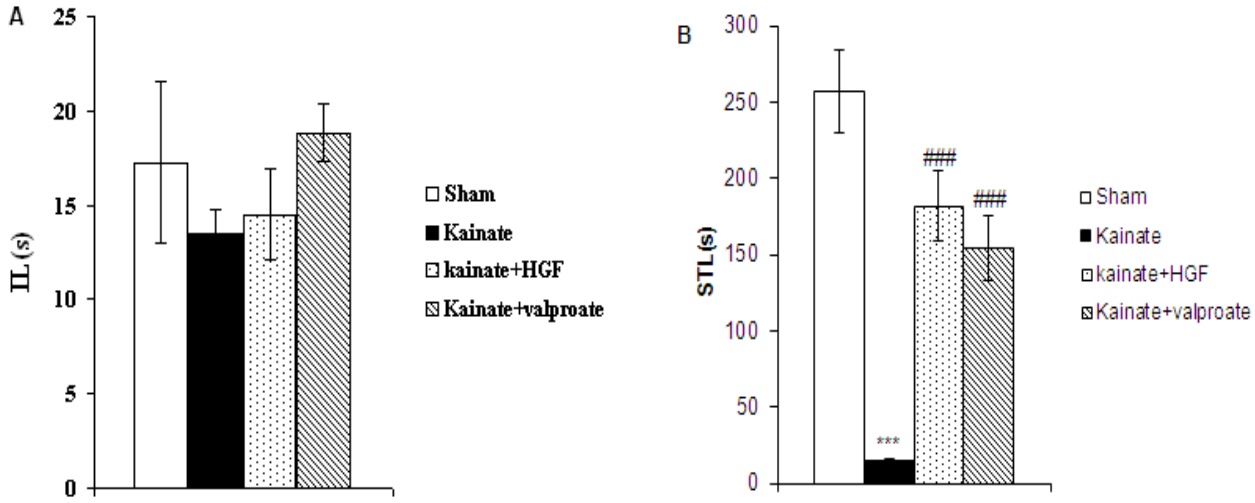

Fig. 2. Initial latency (A) and step-through latency (B) recorded in a single-trial passive avoidance test for rats. Values are means ± SEM
*** $p< 0.001$ (vs. Sham); ### $p < 0.001$ (vs. Kainate)

4. Discussion

In this study, the effect of HGF on learning and memory abilities in a rat model of kainic acid–induced epilepsy was evaluated. The main findings were as follows: two weeks post-surgery, the kainic acid-injected rats showed a considerable increase in Racine's seizure scores and administration of HGF significantly decreased these scores and alternation percentage and STL in the kainic acid-injected rats decreased and treatment of kainate rats with HGF increases their alternation percentage and STL.

According to the obtained results, it seems that administration of HGF could improve epileptic seizures and learning and spatial recognition memory in kainic acid-injected rats. As we know, the local GABAergic neurons interfere the action of excitatory neurons in cortical neuronal circuits (20). Disturbance in these inhibitory pathways leads to establishment of inappropriate neuronal circuits and development of seizures in human (21-23). Hepatocyte growth factor/scatter factor (HGF/SF) is a growth factor with several functions that produced by stromal cells. Phosphorylation of HGF by tyrosine kinase activates the signaling pathways via its c-Met receptor. It has been reported that HGF has functional roles in differentiation, proliferation, organogenesis (24), and regeneration of different types of cells (25-27). In human, the presence of HGF/SF in the brain is necessary for endurance of motor and sensory neurons (28-30). In antenatal forebrain, hepatocyte growth factor/scatter factor (HGF/SF) is expressed and controls migration of neurons (31). According to a recent study, it has been revealed that in Parkinson's disease (32), the function of HGF is disturbed. Also, activation of HGF/SF by serine proteases such as urokinase type plasminogen activator (uPA) (33) has essential role in HGF functions. Binding of uPA to its receptor speeds up the protease activity (34). Gradual decline in

amount or activity of receptor of uPA leads to spontaneous seizures through decreasing HGF/SF in the forebrain and interneuron failure (35, 31).

Recent studies has shown that in mouse, a post-natal decrease of HGF/SF causes deficiency in the GABAergic interneuron function and neural circuit formation. Thus, treatment of animals by HGF/SF returns the GABAergic interneurons of the parietal cortex to its original or usable and functional condition. This returning of the GABAergic interneuron function to normal conditions cause decreases considerably the spontaneous seizures. It seems that the deficiency in the receptors of urokinase type plasminogen activator due to HGF/SF perturbation plays a main role in the disturbance of GABAergic interneurons of the cerebral cortex and increased their sensitivity to chemically induced seizure and irregular intracortical EEG recordings (35).

In this study, treatment of the kainate-induced epileptic rats with HGF attenuated the kainic acid-induced seizure scores and memory and learning inability. It is suggested that HGF decreases the susceptibility of GABAergic interneurons to kainic acid and restores the normal function of GABAergic interneurons. It is obvious that more studies are necessary for elucidating the involved mechanisms of beneficial effects of HGF in the rat model of kainate-induced epilepsy.

To conclude, **t**his study revealed that HGF administration to kainate-injected rats attenuates seizure scores and improves learning and memory.

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