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Short communication

Erythropoietin prevents cognition impairment induced by transient brain ischemia in gerbils

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Abstract

Erythropoietin has recently been studied for its role in the central nervous system (CNS). It has been shown to exert neuroprotective effects in different models of brain injury. We studied whether neuroprotective effects assessed from the reduction of neuronal loss after transient brain ischemia are associated to the preservation of learning ability. Recombinant human erythropoietin (0.5–25 U) was injected in the lateral cerebral ventricle of gerbils that are subjected to temporary (3 min) bilateral carotid occlusion. Post-ischemic histological evaluation of CA1 area neuronal loss and passive avoidance test were performed. Treatment with recombinant human erythropoietin significantly reduced delayed neuronal death in the CA1 area of the hippocampus and prevented cognition impairment in the passive avoidance test. These data indicate that recombinant human erythropoietin neuroprotective effects in brain ischemia are associated with the preservation of learning function. © 2002 Published by Elsevier Science B.V.

Keywords: Erythropoietin; Brain ischemia; Passive avoidance; Learning ability; (Gerbil)

1. Introduction

Erythropoietin is a growth factor that regulates human erythropoiesis (Jelkmann, 1992; Kendall, 2001). It is mainly produced by the kidney, while the liver is more active in erythropoietin synthesis in the human fetus (Dame et al., 1998). The gene of erythropoietin is widely expressed in the bone marrow, spleen, gastrointestinal tract, heart, lung, gonads, and the central nervous system (CNS) (Juul et al., 1998). Recently, scientific interest was turned to the possible role of the hormone in CNS and the presence of receptors for erythropoietin in the nervous cells has been shown (Digicaylioglu et al., 1996; Yamaji et al., 1996). In the CNS, the erythropoietin gene is expressed in the temporal cortex, amygdala, and hippocampus (Marti et al., 1996; Morishita et al., 1997).

It has been hypothesized that erythropoietin and its receptor are prominent in the brain during fetal development, leading to speculation that they play an important role in neurodevelopment (Juul, 2000). Studies with various experimental models of brain ischemia and in vitro evidence pointed to a neuroprotective role for erythropoietin (Sadamoto et al., 1998; Buemi et al., 2000). Possible mechanisms suggested to be involved in erythropoietin protection against brain ischemic injury include activation of specific protein kinases (Sirén et al., 2001) and the inhibition of nitric oxide overproduction occurring after brain ischemia (Calapai et al., 2000).

Cerebral ischemia produces memory dysfunction in rodents (Hirakawa et al., 1994). In particular, it has been observed that short periods of transient brain ischemia in the Mongolian gerbil produce a weakening of learning ability (Karasawa et al., 1994). In the light of these findings, the aim of the present study was to investigate in vivo if the protection by erythropoietin against neurodegeneration is associated with the preservation of learning ability, as evaluated with the passive avoidance test in Mongolian gerbils subjected to transient cerebral ischemia.

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2. Materials and methods

2.1. Animals

Adult male Mongolian gerbils weighing 60–70 g were used. Before and after ischemia/reperfusion, they were housed four to a cage at a constant room temperature of 21–22 °C under a light cycle of 12/12 h (7:00 a.m./7:00 p.m.). The animals were allowed free access to food and drinking water. Adaptation and experiments were carried out in accordance with the internationally accepted principles and the national laws concerning the care and use of laboratory animals and were approved by the Ethical Commitee of the University of Messina.

2.2. Treatment

Seven days before the experiments, the gerbils were anesthetized with chloral hydrate, placed in a stereotaxic apparatus and a guide cannula was implanted into the left lateral ventricle, according to the atlas of Thiessen and Yahr (1977). Animals subjected to brain ischemia, or sham animals, were treated in the left lateral cerebral ventricle with artificial cerebrospinal fluid (vehicle) or with recombinant human erythropoietin (0.5–25 U) immediately after restoration of blood flow.

2.3. Surgery

The ischemia/reperfusion injury was induced by a single 3-min bilateral occlusion of the common carotid arteries initially (about 4–5 min) under halothane (5%) anesthesia followed by nitrous oxygen/O_2 (70% N_2O/30% O_2); during surgery, halothane was decreased to 2%. Adequacy of the anaesthesia was monitored by foot pinch. The total period of anesthesia was about 18 min. Sham-operated animals, not exposed to ischemic insult, served as controls. Body temperature was maintained at 36–37 °C during the ischemic and the immediate post-ischemic period with a homeothermic blanket. Surgery was always done between 10:00 and 12:00 a.m.

2.4. Passive avoidance

The gerbils were trained in a step-through type passive avoidance apparatus 7 days after brain ischemia. The passive avoidance apparatus is divided into two sections. One compartment is white and illuminated by a light, set on the lid, the other compartment is dark and without illumination. The two compartments communicate through a sliding-door system and the floor is a steel grid in both compartments. The bars of the dark compartment floor are wired to a constant current scrambler circuit (Misane et al., 1998).

The experimental session was divided into three phases: habituation trial, acquisition trial, and retention trial (Otano et al., 1999). During the habituation trial, the gerbil is placed in the white and illuminated compartment. In this phase, the sliding doors are initially closed and they open after 3 s. The gerbil can now explore both compartments for 90 s and, after this period, it is taken off the apparatus. After 12 min, it is placed again in the white compartment. The sliding doors open after 3 s and successively close when the gerbil crosses the cage, entering the dark room, where it remains for 10 s, then is removed from the cage. The acquisition trial is performed 60 min after the habituation trial. In this phase, the gerbil is replaced in the white room and when it crosses the sliding doors entering the dark room, it receives an electric shock (4 mA for 5 s), released from the grid. Twenty-four hours later, the retention trial is performed. The gerbil is replaced in the white room and the sliding doors open as in the previous phases after 3 s. During this phase, a timer measures the response latency as the period, in seconds, between the time when the gerbil is placed in the white room and the moment when the animal crosses in the dark compartment. The cut-off time was set at 300 s.

2.5. Delayed neuronal death

After the passive avoidance test, the animals were killed by decaptation under anesthesia with chloral hydrate. The brain was dissected out from the skull and preserved in 10% buffered neutral formalin for 10 days. Formalin-fixed brain blocks containing dorsal hippocampus were embedded in

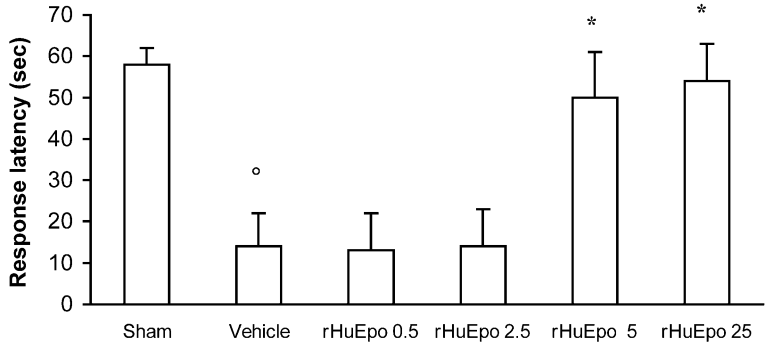

Fig. 1. Effects of i.c.v. administration of recombinant human erythropoietin (0.5–25 U) on response latency (seconds) in the passive avoidance test after transient bilateral carotid occlusion in gerbils. Passive avoidance was tested 7 days after brain ischemia. Each column represents the mean ± S.E. for five to six animals. * $P<0.01$ vs. vehicle; ** $P<0.001$ vs. vehicle.

paraffin. Thick sections (5 μm) were sliced at the level of the dorsal hippocampus and stained with hematoxylin and eosin for light microscopic examination. The grading system of Pulsinelli et al. (1982) was used: grade 0 = 0% of the neurons damaged (normal brain), 1 = 1% to 10% of the neurons damaged, 2 = 11% to 50% of the neurons damaged, 3 = more than 50% of the neurons damaged, and 4 = infarction (necrosis of both neurons and glia). The slices were evaluated independently by two observers.

2.6. Statistical analysis

All statistical procedures were performed using SPSS statistical software package release 6.1.3 (SPSS, Chicago, IL, USA). Data analysis was performed using one-way analysis of variance (ANOVA) with the Scheffè posthoc test for multiple comparisons. The data are expressed as the means ± S.E.M. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Passive avoidance

In the passive avoidance test, the gerbils subjected to brain ischemia and treated with vehicle showed a reduced response latency compared to that of sham animals (Fig. 1). These data indicate that ischemia reduced the ability to learn the information provided during the test.

In animals treated with the highest doses of erythropoietin (12.5 and 25 U), the response latency was similar to that of sham animals (Fig. 1).

3.2. Delayed neuronal death

Animals subjected to transient brain ischemia and treated with recombinant human erythropoietin showed a significant and dose-dependent reduction of the histological score obtained from histological examination compared to that of gerbils treated with vehicle (Fig. 2).

4. Discussion

Erythropoietin may be implicated in the development of the CNS (Juul, 2000) and in the neuroprotective mechanisms triggered by cerebral insults (Sakanaka et al., 1998). In a previous work, in agreement with other authors (Sirén et al., 2001), we observed that systemic or central administration of erythropoietin has beneficial effects when given to animals with experimental models of brain ischemia. However, while central administration produces neuroprotective effects when given either before or after ischemia, systemic administration of erythropoietin is effective only if done after the ischemic event (Calapai et al., 2000). The reason for this difference could be that it is unlikely that erythropoietin crosses a normal blood–brain barrier and that there is a possibility that the hormone, when given systemically, can cross a blood–brain barrier made dysfunctional by ischemia itself (Dame et al., 2001). We have administered erythropoietin immediately after the ischemic event at the moment of reopening of both carotids and only through a central route of administration.

It is well known that transient brain ischemia, induced by bilateral carotid occlusion in Mongolian gerbils, hits selectively neurons in the CA1 region of the hippocampus (Ito et al., 1975). This area of the CNS is related with memory and habituation, and damage to these neurons causes behavioral abnormalities that can be revealed by means of laboratory tests (O'Keefe and Nadel, 1978; Will et al., 1986).

Passive avoidance testing was performed 7 days after ischemia and animals subjected to transient bilateral occlusion had a response latency shorter than that of sham animals, indicating an impairment of learning ability. Data obtained with erythropoietin-treated animals show a response latency similar to that of sham animals, indicating that erythropoietin action can interfere with functional effects of brain ischemic injury on learning ability.

Neuronal loss, caused by short periods of ischemia, can be observed a few days after the ischemic event because neurons of CA1 area are particularly sensitive to short periods of ischemia and show a delay in morphological cell

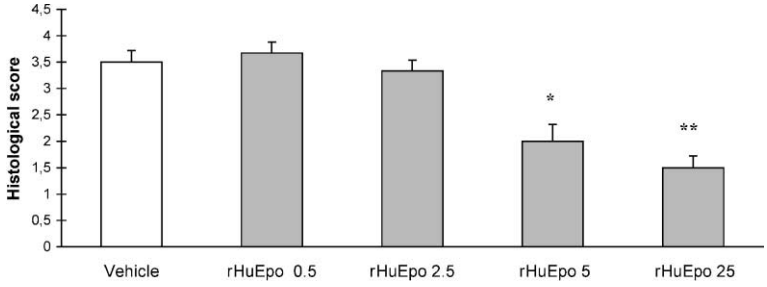

Fig. 2. Effects of i.c.v. administration of recombinant human erythropoietin (0.5–25 U) on histological evaluation of delayed neuronal death in the CA1 area after transient bilateral carotid occlusion in gerbils. Each column represents the mean ± S.E. for five to six animals. °$P < 0.001$ vs. sham; *$P < 0.01$ vs. vehicle; **$P < 0.001$ vs. vehicle.

death (delayed neuronal death) (Kirino, 2000). The finding of reduction of the histological score that was used to evaluate neuronal loss shows that eryhthropoietin produced partial protection of the CA1 area from neuronal degeneration. Hippocampal CA1 neurons are important components of a network involved in the learning processes. So, it is possible to hypothesize about a relation between erythropoietin neuroprotective effects and an abolition of a learning deficit induced by ischemic damage.

Our data suggest that the neuroprotection induced by recombinant human erythropoietin in transient brain ischemia can oppose the appearance of functional abnormalities such as learning inability.

Several lines of evidence indicate that this substance may interact with compounds or mechanisms implicated in brain ischemia. However, the exact mechanism of action of erythropoietin neuroprotection is still not clear. In spite of this incomplete knowledge of the basis of the neuroprotection provided by erythropoietin, these data confirm that the hormone known mainly for its hematopoietic function is also a candidate for a key role in the brain.

In the light of these findings we can suggest that neuroprotective effects in experimental animals, also observed by other authors, have beneficial consequences, leading to the possibility that erythropoietin treatment could induce similar results in humans affected by learning inability subsequent to cerebral ischemic injury.

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