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# Histogranin reduced brain injury after transient focal ischemia in rats

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**Abstract**

Excitatory amino acids (EAAs) play an important role during ischemic brain injury. In this study we examined the protective effect of histogranin (HN), an endogenous peptide that antagonizes excitatory amino acids-mediated activity noncompetitively, in an animal model of cerebral ischemia. Adult rats were anesthetized with chloral hydrate. Histogranin was given intracerebroventricularly before a 60-min middle cerebral artery occlusion (MCAo). Animals were examined for their locomotor activity 2 days after MCAo. Histogranin significantly increased locomotor activity in the stroke rats. Histogranin pretreatment reduced the volume of cerebral infarction and the caspase-3 immunoreactivity in the stroke animals. Taken together, our data suggest that histogranin is protective against ischemic brain injury. The protective effect may involve anti-apoptotic mechanisms. Published by Elsevier Ireland Ltd.

*Keywords:* Glutamate; Histogranin; Neuroprotection; Stroke

Histogranin (HN) is an endogenous peptide (Met-Asn-Tyr-Ala-Leu-Lys-Gly-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe) with a structure 80% homologous with a fragment-(86–100) of histone H4. It is concentrated in the pituitary, adrenal glands, and spleen. It is also found in brain and blood plasma at lower levels. Previous studies have indicated that HN, when given intracerebroventricularly, antagonized *N*-methyl-D-aspartate (NMDA), but not AMPA, kainite or bicuculline, -mediated convulsions [8]. HN and related peptides specifically enhance the number of [$^3$H]dextromethorphan binding sites associated with the NMDA receptor complex [4]. It also non-competitively inhibited the binding of [3H]CGP-39653, a specific NMDA receptor ligand, to membrane preparations of rat brain [19]. These data suggest that HN may serve as an endogenous antagonist for the *N*-methyl-D-aspartate (NMDA) receptor.

Excitatory amino acids (EAAs) have been found to contribute to damage in ischemic brain injury in animal study. Cerebral ischemia induces glutamate release, decreases glutamate uptake [2,20], and activates EAA receptors [15,12]. Systemic application of glutamate transporter inhibitors or deletion of glial glutamate transporter gene GLT-1 enhance ischemic brain injury [14]. Blockade of glutamate production [21], release, or its interaction with EAA receptors using synthetic antagonists, reduce cerebral damage [6,9,10]. For example, pretreatment with ketamine, an EAA antagonist, reduced nitric oxide release from cerebral cortex [10] and neuronal loss in the ischemic cortex [16]. MK801 and other synthetic glutamate antagonists reduced hypoxia/reoxygenation-mediated injury in primary cortical neurons [5] as well as ischemia/hypoxia-mediated caspase-3 activation [17] and cerebral damage [1,13,22,25]. These data suggest that cerebral ischemia increases synaptic glutamate levels and reducing EAA transmission with exogeneous EAA antagonists can reduce ischemic brain damage in animal models of stroke. The interaction of ischemia and endogenous EAA antagonist, however, is not known.

In this study, we examined the effects of HN in a rodent model of ischemia. Adult male Sprague–Dawley rats (Charles River Laboratory Inc.) were anesthetized with chloral hydrate (400 mg/kg, i.p.). HN (30 nmol in 10 μL) or vehicle (saline, 10 μL) was administered into the lateral ventricle in 4 min through a Hamilton syringe before middle cerebral artery occlusion (MCAo). The co-ordinates were: 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline; 3.7 mm below dura surface. Ten to 15 min after i.c.v. administration, animals were subjected to cerebral ischemia. The ligation of the right MCA and bilateral common carotids (CCAs) was performed using

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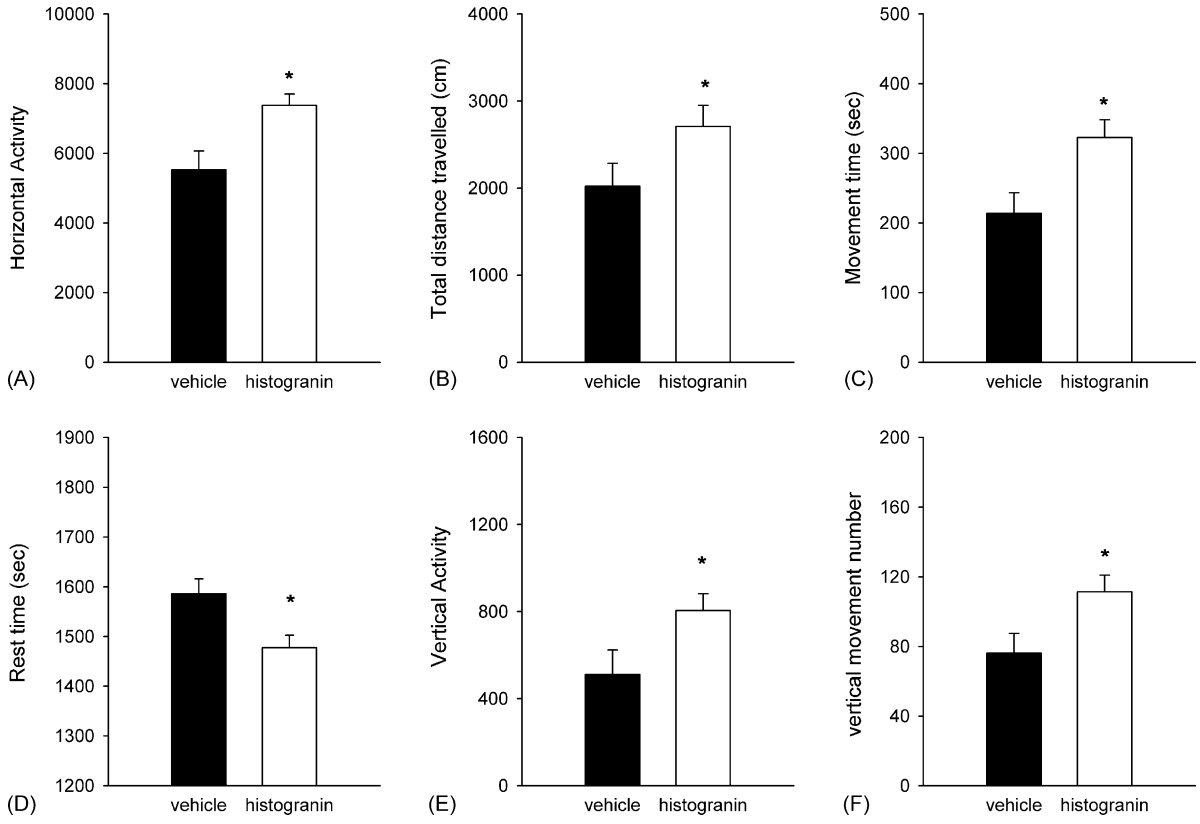


Fig. 1. Pretreatment with histogranin (HN) improved locomotor activity in stroke animals. Animals were pretreated with HN or vehicle intracerebroventricularly before a 60-min distal MCAo. All animals were placed in locomotor activity chambers for 30 min at 2 days after MCAo. Rats that received HN, compared to vehicle, had a significant increment in (A) horizontal activity, (B) total distance traveled, (C) movement time, (E) vertical activity, and (F) vertical movement number. There is a significant decrease in rest time (D) in rats pretreated with HN. $^{*}p < 0.05$, $t$ test.

methods previously described [3,23]. The CCAs were clamped with non-traumatic arterial clips. The right MCA was ligated with 10-O suture. The ligature and clips were removed after 60-min ischemia to allow reperfusion. Core body temperature was monitored with a thermistor probe and maintained at 37 °C with a heating pad during surgery. After surgery, the animals were kept in a temperature-controlled incubator to maintained body temperature at 37 °C.

We first examined if HN can improve locomotor behavior in stroke rats. Animals (HN: $n = 8$; vehicle, $n = 10$) were placed in an Accuscan activity monitor (Columbus, OH) 2 days after MCAo. The monitor contained 16 horizontal and 8 vertical infrared sensors spaced 2.5 cm apart. The vertical sensors were situated 11.5 cm from the floor of the chamber. Each animal was placed in a 42 cm × 42 cm × 31 cm plexiglass open box for 30 min. Motor activity was calculated using the number of beams broken by the animals. All stroke animals developed a significant decrease in locomotor activity. In animals pretreated with HN, compared to those treated with vehicle, there was a significant increase ($p < 0.05$, $t$ test, Fig. 1) in (A) horizontal activity (total number of beam interruptions that occurred in the horizontal sensor), (B) total distance traveled, (C) horizontal movement time (the amount of time the animal was in ambulation), (E) vertical activity (the total number of beam interruptions that occurred in the vertical sensor), and (F) vertical movement number (the number of vertical movement executed by the animal). On the other hand, the animals receiving HN pretreatment had significantly less rest time during 30 min recording period (Fig. 1D, $p < 0.05$, $t$ test).

The volume of cerebral infarction was examined 2 days after MCAo. Animals (HN: $n = 8$; vehicle, $n = 10$) were euthanized and brain tissue was sliced into 2.0 mm thick sections. The brain slices were incubated in 2% (w/v) triphenyltetrazolium chloride (TTC, Sigma) for 10 min at room temperature, and then transferred into a 4% (w/v) paraformaldehyde solution for fixation. The area of infarction was measured double blind using a digital scanner and the Image Tools program (University of Texas Health Sciences Center, San Antonio). Typical TTC staining is demonstrated in Fig. 2A. The total infarction volume in each animal was obtained from the product of average slice thickness (2 mm) and sum of the area of infarction in all brain slices. We found that the volume of infarction was significantly reduced by pretreatment with HN (Fig. 2B, $p < 0.05$, $t$ test). Taken together, these data suggest that HN improves locomotor behavior and reduces cerebral infarction in stroke rats.

Physiological parameters were measured in 16 rats as previously described [24]. Animals were anesthetized with chloral hydrate and a polyethylene catheter was inserted into the right femoral artery. Arterial blood (<1 mL) was withdrawn 20–30 min after the intracerebroventricular administration of HN or vehicle (10 μL). Blood gases were analyzed using standard methods. We found that pretreatment with HN did not