LETTER TO THE EDITOR

Ghrelin Attenuated Cerebral Ischemia Reperfusion Injury in Rats

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Ghrelin is a novel 28-amino acid peptide with an n-octanoyl ester in the serine 3 that has recently been discovered in rat and human gastrointestinal tract, as an endogenous ligand for growth hormone (GH) secretagogue receptor [1]. Ghrelin is produced and stored in granules of the special neuroendocrine cells (A-like cells), located mainly in oxyntic mucosa [2]. Ghrelin stimulates GH release and induces a positive energy balance by stimulating food intake and decreasing fat use [3]. Ghrelin also has numerous peripheral actions including direct effects on exocrine and endocrine pancreatic functions, carbohydrate metabolism, gastric secretion, stomach motility, and sleep [4].

Recently, some authors revealed that administration of ghrelin during reperfusion had protective effects on myocardial ischemia reperfusion injury, including reduced ischemic volume, improvements in coronary flow, and attenuation of lipid peroxidation in a rat model [5]. But the exact effect of ghrelin on focal cerebral ischemia reperfusion and the mechanism underlying it are still unknown.

Focal cerebral ischemia in adult male Sprague-Dawley rats (weighing 250–280 g) was induced by MCAO (middle cerebral artery occlusion) for 90 min with an intraluminal filament according to the method previously described by Longa [6]. Rat ghrelin was administered intraperitoneally (160 µg/kg) 30 min before MCAO and at the beginning of reperfusion. Brain samples were obtained 24 h after MCAO. In this study, we demonstrated that systemic administration of ghrelin significantly reduced infarct volumes from MCAO (data shown in Figure 1A). The protective effect of ghrelin on cell apoptosis and neuronal degeneration after ischemia reperfusion injury was evaluated by TUNEL and Fluoro-Jade B staining, a kind of polyanionic fluorescein derivative that specifically bound to degenerating neurons. In ghrelin-treated group, the number of TUNEL-positive cells and degenerating neurons in the

peri-infarct area decreased significantly (Figure 1B,C). Given the fact that ghrelin can pass through the blood–brain barrier [7], these findings suggest that peripherally administered ghrelin enters the brain parenchyma and exerts neuroprotective effects on cerebral ischemia reperfusion. The inhibitive effect of ghrelin on apoptosis was supposed to be related to the activation of PI3K/AKT signaling pathway by the phosphorylation of Akt in an endothelial cell injury [8].

Reactive oxygen species (ROS) have been implicated in the pathogenesis of cerebral ischemia reperfusion injury [9]. These oxygen-free radicals are directly toxic to neurons and initiate a free radical-mediated cascade causing neuronal damage. To assess the effect of ghrelin on ROS, we evaluated the production of superoxide anion, an important reactive oxygen species (ROS) using DHE (dihydroethidium). In this study, we found superoxide anion production was enhanced after reperfusion, and ghrelin treatment could attenuate it (Figure 2). These might be related to the mechanism that ghrelin could inhibit NADPH oxidase activation and reduce ROS production [10].

In summary, we have demonstrated that peripheral administration of ghrelin could protect neurons from ischemic injury caused by MCAO. The protective effects of ghrelin appear to be related with an inhibition of apoptosis and oxidative stress. Our results suggest that ghrelin can function as a neuroprotective agent, and administration of ghrelin may be a novel therapeutic strategy for the treatment of ischemic stroke.

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Figure 1 Ghrelin treatment attenuated ischemic brain damage. **(A)** The infarct volumes examined by TTC staining of control group rats were $285 \pm 38.6 \text{ mm}^3$, whereas the volumes of ghrelin-treated groups were $229 \pm 20.2 \text{ mm}^3$ ($P < 0.01$). **(B)** The number of TUNEL-positive cells observed in the peri-infarct area at 24 h after ischemia in ghrelin group was $15.8 \pm 5.1/10,000 \mu\text{m}^2$, which was significantly less than that in control group $22.6 \pm 6.5/10,000 \mu\text{m}^2$ ($P < 0.05$). **(C)** In ghrelin group, the number of degenerating neurons stained by Fluoro-Jade B in the peri-infarct area 24 h after ischemia was $15.1 \pm 5.5/10,000 \mu\text{m}^2$, but $26.2 \pm 4.5/10,000 \mu\text{m}^2$ in control group ($P < 0.05$). Data were expressed as mean $\pm$ SD ($n = 6$ in each group). Data were analyzed by one-way ANOVA with Newman Keuls post hoc test.

Figure 2 Ghrelin treatment attenuated superoxide production after ischemia reperfusion. The intensity of fluorescence (arbitrary unit) (4820 ± 885) in the ischemic areas of ghrelin treated rats was statistically different from that (3150 ± 690) in control group (** $P < 0.01$). Data were expressed as mean $\pm$ SD ($n = 6$ in each group). Data were analyzed by one-way ANOVA with Newman Keuls post hoc test.

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