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The protective effects of calcitonin gene-related peptide on gastric mucosa injury after cerebral ischemia reperfusion in rats

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ABSTRACT

High is the incidence of gastrointestinal dysfunction induced by cerebrovascular disease. However, little is known about the effects of CGRP on gastrointestinal injuries induced by cerebrovascular disease. The purpose of the present study was to investigate the protective effects of calcitonin gene-related peptide (CGRP) on gastric mucosa injury after focal cerebral ischemia reperfusion in rats. Thirty healthy adult male Wistar rats were selected for this experiment and were randomly divided into CGRP-treated, sham-operated, and control groups, respectively. Ten rats were involved in each group. Focal cerebral ischemia reperfusion rat model was established by a 2-hour left middle cerebral artery occlusion (MCAO) using an intraluminal filament, followed by 46 h of reperfusion. CGRP (1 μg/ml) at the dose of 3 μg/kg was injected intraperitoneally (i.p.) at the beginning of reperfusion for rats in CGRP-treated group. Saline as vehicle (3 ml/kg body weight), i.p., was administered at the beginning of reperfusion for rats in control group. Sham-operated animals were subjected to an operation without MCAO. Forty-eight hours after operation, the samples were taken out and processed for calculating stomach mucous membrane damage index according to Guth method, detecting pathological changes of gastric mucosa tissue by light microscopy, determining mast cell distribution by toluidine blue staining, and observing the expression of gastrin (Gas), somatostatin (SST), aquaporin-4 (AQP4), and basic fibroblast growth factor (bFGF) by immunohistochemical staining. The results showed that: (1) Gastric mucosa with diffuse edema, splinter hemorrhage and erosion, numerous endothelial cells necrosis, mucosa dissociation, infiltration of inflammatory cells were observed in both control and CGRP-treated animals. CGRP administration could reduce the damage of gastric mucosa. The injury index of gastric mucosa was lower in CGRP-treated group as compared with that in control group ($P<0.05$). (2) Gas expression in gastric antrum mucosa was lower in CGRP-treated group than that in control group ($P<0.01$). SST expression in gastric antrum mucosa was higher in CGRP-treated group than that in control group ($P<0.01$). AQP4 expression in gastric mucosa was lower in CGRP-treated group than that in control group ($P<0.05$). bFGF expression in gastric mucosa was higher in CGRP-treated group than that in control group ($P<0.01$). (3) The mast cell degranulation ratio in control group in gastric mucosa was significantly higher than that in CGRP-treated group ($P<0.01$). It is concluded that CGRP can regulate the secretion of Gas, SST, AQP_4, and bFGF, inhibit mast cell degaranulation and thus alleviate the damage of gastric mucosa induced by cerebral ischemia and reperfusion. CGRP may be one of the good candidates of potential clinical therapy drugs for regulating gastric mucosal protection and maintaining gastric mucosal integrity after cerebral ischemia and reperfusion.

1. Introduction

Cerebrovascular disease is one of the common diseases that most compromise human spices [1]. The onset of the cerebrovascular disease induces several organs injuries, even leading to failure of organs [2]. High is the incidence of gastrointestinal dysfunction induced by cerebrovascular disease, which in return aggravates the original brain injury. Hence, the approach of searching for both cerebral and gastrointestinal protective drugs is of great importance.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide produced by tissue-specific alternative splicing of the primary transcript of the calcitonin/CGRP gene [3]. CGRP is one of the brain–gut peptides widely distributed in the central and peripheral

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nervous system and exhibits numerous biological activities in mammals [3–5]. CGRP is one of the most potent endogenous vasodilators known [6–8] and regulates the physiolocial and pathological function of brain–gut axis [9]. CGRP seems to be the predominant neurotransmitter of spinal afferents in the rat stomach exerting many pharmacological effects by direct or indirect mechanisms [10]. It has been shown that CGRP might protect gastric mucosa under stress conditions [11]. CGRP positive nerve fibers also appeared in the gastric mucosa and muscle layers [12]. The ability of the adaptation to repeated exposure to cold restraint stress of the stomach is partially mediated by endogenous CGRP expressed in capsaicin-sensitive sensory nerves [13]. Stimulation of sensory nerves with the expression and release of CGRP appears to aid the restoration of mucosal repair and microcirculation in gastric ulcer bed [14]. Gastric tissue CGRP levels were transiently increased in rats with water immersion restraint stress-induced gastric mucosal injury [15]. CGRP production increase in sensory neurons plays a critical role in the reduction of stress-induced gastric mucosal injury [16,17]. Increased release of CGRP may contribute to gastroprotective and anti-acid secretory effect [18]. CGRP is one of the gastrointestinal mucosa regulatory neuropeptides [19] and thought to be involved in the regulation of gastric blood flow and in the control of gastric acid secretion [20].

Interestingly, CGRP is also known as an endogenous protection substance generated by gastric epithelial cells [21]. Clinical report suggested that CGRP had effects on the disease of digestive tract [22]. Increase of CGRP release from gastric tissues is related to gastroprotection [23]. Enhancement of gastric mucosal expression of the CGRP gene and elevation of CGRP serum levels may produce gastroprotective effect against acute gastric mucosal damage induced by ethanol and water immersion restraint stress [9,24–28]. Endogenous CGRP can prevent gastric mucosal injury elicited by ethanol and enhance healing of acetic acid-induced ulcer using CGRP knockout mice [29]. It has been suggested that CGRP receptors are expressed in D cells in the rat gastric mucosa [30]. Blockade of CGRP receptor could inhibit gastroprotective effect of other agents which are beneficial for prevention or therapy on gastric mucosal damage [15,31–40]. Interestingly, intracerebroventricular injection (i.c.v.) of CGRP antagonist could inhibit vanilloid receptor 1 (VR1)-mediated regulation of gastric acid secretion in the rat brain regions suggesting CGRP-related brain–gut axis is involved in the gastroprotection [41]. Furthermore, brain–gut axis in gastroprotection by other agents was significantly attenuated by CGRP antagonist [42–44].

However, little is known about the effects of CGRP on gastrointestinal injuries induced by cerebrovascular disease. In the present study, experiments were carried out to investigate the protective effects of CGRP on gastric mucosa injury after cerebral ischemia reperfusion in middle cerebral artery occlusion (MCAO) rat models.

2. Materials and methods

2.1. Experimental animals and MCAO model establishment

Thirty healthy adult male Wistar rats, obtained from the Experimental Animal Center of Shandong University of China, weighing 280–320 g, were randomly divided into CGRP-treated, sham-operated, and control groups, respectively. Ten rats were involved in each group.

All rats, fed on a pellet diet and given water freely, were kept in the animal unit at least 2 days prior to the experiments and were fasted for 12 h before the expriments, but were given free access to water. All the experiments were conducted according to local institutional guidelines for the care and use of laboratory animals. The animals were anesthetized with 3.5% chloral hydrate solution (1 ml/100 g, i.p.). MCAO was induced by intraluminal filament method as described previously. Briefly, the left common carotid artery (CCA) and the external carotid artery (ECA) were exposed. Then, a 0.2 mm in diameter nylon suture was carefully inserted from the ECA into the internal carotid artery (ICA) and was advanced towards to occlude the origin of the left middle cerebral artery (MCA) until a light resistance was felt (18–20 mm from CCA bifurcation). After 2 h of MCAO, the nylon suture was withdrawn, followed by 46 h of reperfusion.

CGRP (1 μg/ml) at the dose of 3 μg/kg or saline (3 ml/kg body weight), i.p., was administered at the beginning of reperfusion in CGRP-treated and control animals, respectively. Animal body temperature was maintained at 37 ± 1 °C during and after the operations. The sham-operated animals involved underwent the same surgical procedure without inserting the nylon suture.

2.2. Gastric mucosa injury index detection

Rats were anesthetized with chloral hydrate solution (3.5%, 1 ml/100 g, i.p.). The stomach was taken out, cut along the arcus major ventriculi, and rinsed by saline with the mucosa turned outside. The samples were observed under stereomicroscope with 10× magnification. The gastric mucosa injury index was counted according to Guth grade standard: Spot erosion grade 1; the length of erosion less than 1 mm grade 2; 1–2 mm length of erosion grade 3; 2–3 mm length of erosion grade 4, more than 4 mm length of erosion grade 5; and the width more than 1 mm is multiplied by 2. The gastric mucosa injury index was described as mean ± standard deviation ($\bar{x} \pm SD$).

2.3. Stomach histological observation

Gastric tissue of antrum and partial corpus ventriculi in 1 cm length and 0.5 cm width was cut along arcus minor ventriculi, submerged into 4% paraformaldehyde for 12 h fixation, rinsed by current water for 12 h, dehydrated in gradient alcohol, paraffin-embedded, cut into consecutive 4 μm-thick slices, stained with HE method and observed under microscope.

2.4. Immunohistochemical staining

PV-6001 two-step immunohistochemistry was employed after deparaffinage. Briefly, the sections were incubated in 3% hydrogen peroxide in methanol for 10 min at room temperature and antigen-retrieved by microwave. After being washed 3 times for 5 min with 0.01 mol/L PBS buffer, the sections were blocked with 10% goat serum for 10 min at room temperature. Then the sections were incubated with rabbit anti-SST IgG (1:100, Boster) or rabbit anti-Gas polyclonal IgG (1:200, Thermo), rabbit anti-bFGF IgG (1:400, Chemicon) or rabbit anti-AQP4 polyclonal IgG (1:200, Sigma) overnight at 4 °C. After being washed three times for 10 min with PBS, the sections were incubated with goat anti-rabbit IgG antibody-HRP polymer at 37 °C for 30 min. Reaction product was detected using 3,3′-diaminobenzidinetetrahydrochloride (DAB). Negative control was performed by replacing the primary antibody with PBS. Sections were dehydrated through graded alcohols, cleared in xylene and coverslipped in neutral gum. Five different visual fields randomly selected in each slice were observed and the SST, Gas, bFGF and AQP4 positive cells were analyzed by IMAGE-PRO PLUS. The data were expressed as the percent of gray-scale value of control group. The positive G cells and D cells were counted.

2.5. Mast cells counting and degranulation detection

After deparaffinage, the sections were cut into 4 μm thick slices, submerged into toluidine blue for 5 h at normal temperature, dehydrated by acetone, transparentized by xylene, and sealed by neutral gum. Mast cells in mucosa and submucosa each consecutive slice was counted in 10 visual fields 400×, and the result was expressed as number/visual field. The degranulated mast cell ratio was expressed as the number of degranulated mast cells/the total number of mast cells.