Pharmacological Research 102 (2015) 276–285

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Peptidic exenatide and herbal catalpol mediate neuroprotection via the hippocampal GLP-1 receptor/β-endorphin pathway

Yu Jia, Nian Gong, Teng-Fei Li, Bin Zhu, Yong-Xiang Wang*

King's Lab, Shanghai Jiao Tong University School of Pharmacy, Shanghai, China

ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 7 October 2015
Accepted 12 October 2015
Available online 3 November 2015

Keywords:
Glucagon-like peptide-1 receptor (GLP-1R)
β-endorphin
Microglia
Neuroprotection
Exenatide
Catalpol

ABSTRACT

Both peptidic agonist exenatide and herbal agonist catalpol of the glucagon-like peptide-1 receptor (GLP-1R) are neuroprotective. We have previously shown that activation of spinal GLP-1Rs expresses β-endorphin in microglia to produce antinociception. The aim of this study was to explore whether exenatide and catalpol exert neuroprotection via activation of the hippocampal GLP-1R/β-endorphin pathway. The rat middle cerebral artery occlusion model was employed, and the GLP-1R immunofluorescence staining and β-endorphin measurement were assayed in the hippocampus and primary cultures of microglia, neurons and astrocytes. The immunoreactivity of GLP-1Rs on microglia in the hippocampus was upregulated after ischemia reperfusion. Intracerebroventricular (i.c.v.) injection of exenatide and catalpol produced neuroprotection in the rat transient ischemia/reperfusion model, reflected by a marked reduction in brain infarction size and a mild recovery in neurobehavioral deficits. In addition, i.c.v. injection of exenatide and catalpol significantly stimulated β-endorphin expression in the hippocampus and cultured primary microglia (but not primary neurons or astrocytes). Furthermore, exenatide and catalpol neuroprotection was completely blocked by i.c.v. injection of the GLP-1R orthosteric antagonist exendin (9–39), specific β-endorphin antiserum, and selective opioid receptor antagonist naloxone. Our results indicate, for the first time, that the neuroprotective effects of catalpol and exenatide are GLP-1R-specific, and that these effects are mediated by β-endorphin expression probably in hippocampal microglia. We postulate that in contrast to the peripheral tissue, where the activation of GLP-1Rs in pancreas islet β-cells causes secretion of insulin to perform glucoregulation, it leads to β-endorphin expression in microglial cells to produce neuroprotection and analgesia in the central nervous system.

1. Introduction

Glucagon-like peptide-1 receptors (GLP-1Rs) are distributed in various tissues, including the pancreatic islets and lungs, the cardiovascular system, and the central nervous system, but not in the skeletal muscle [1–6]. In the central nervous system, GLP-1Rs are located in the cortex, neocortex, hypothalamus, brainstem, hippocampus, cerebellum, and spinal cord [2,7–12]. GLP-1Rs modulate central neuronal activity and protect against neuronal damage induced by various insults [13–15]. Rapidly accumulating evidence suggests that the GLP-1R agonists exenatide and GLP-1, used for the treatment of type 2 diabetes mellitus, also display neuroprotective properties in multiple models of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyothrophic lateral sclerosis, peripheral neuropathy, multiple sclerosis, ischemia, and stroke [11,14,16–22]. However, the mechanisms and signal transduction underlying the neuroprotective effects of GLP-1R are not clear. It is reported the neuroprotective effects of GLP-1R stimulation on cultured primary cortical neurons, suggesting a neuronal mechanism [14]. In contrast, exenatide was reported to exert neuroprotection via blocking 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced microglial activation and reducing the expression of matrix metalloproteinase-3 and stimulated proinflammatory cytokines (TNF-α and IL-1β) in substantia nigra pars compacta and striatum [21]. Activation of GLP-1Rs was also reported to increase the expression of the proinflammatory cytokines in the hypothalamus and the hindbrain [23], or have no effect on proinflammatory cytokine expression in the spinal cord or cultured primary microglia [24]. On the other hand, exenatide was shown to stimulate neurogenesis, which serves a

Abbreviations: GLP-1R, glucagon-like peptide-1 receptor; MCAO, middle cerebral artery occlusion; TTC, 2,3,5-triphenyltetrazolium chloride; i.c.v., intracerebroventricular; ANOVA, analysis of variance.

* Corresponding author at: King's Lab, Shanghai Jiao Tong University School of Pharmacy, 800 Dongchuan Road, Shanghai 200240, China.
E-mail address: yxwang@sjtu.edu.cn (Y.-X. Wang).

http://dx.doi.org/10.1016/j.phrs.2015.10.008
1043-6618/

possible mechanism by which the peptide might restore function in a damaged system [16,18].

We recently identified the GLP-1R/β-endorphin antinociceptive pathway in the spinal cord. GLP-1Rs were exclusively expressed in the spinal dorsal horn microglial cells, and were consequently upgraded accompanying microglial activation following peripheral nerve injury. The peptidic agonists GLP-1 (7–36) and exenatide, non-peptidic agonist WB4-24, and herbal agonist shanzhiside methylester of GLP-1R produced anti-hypersensitivity in a variety of animal models of chronic pain, including neuropathic pain, inflammatory pain, bone cancer pain, and painful diabetic neuropathy. These GLP-1R agonists stimulated β-endorphin expression in spinal dorsal horn or cultured primary microglial cells from the spinal cord. The antinociceptive effects of exenatide, GLP-1, WB4-24 and shanzhiside methylester were completely blocked by the microglial inhibitor minocycline, specific β-endorphin antiserum (but not the dynorphin A antiserum), and opioid receptor antagonist naloxone or selective μ-opioid receptor antagonist CTAP [2,24,25]. It is not known whether the GLP-1R/β-endorphin pathway also exists in the hippocampus, and whether exenatide produces neuroprotection via this pathway.

Catalpol is an important iridoid glycoside purified from *Rehmannia glutinosa Libosch*, which has been widely used as a traditional Chinese herbal medicine for the treatment of aging diseases and stroke. In the previous studies, catalpol significantly attenuated apoptosis, rescued hippocampal CA1 neurons, and reduced cognitive impairment in the transient focal and global cerebral ischemic models in gerbils [26,27]. It was also recently reported that catalpol significantly facilitated neurological function recovery, reduced infarction volume, and increased cerebral blood flow in a mouse stroke model [28]. It improved memory and protected the forebrain neurons from neurodegeneration in a neurodegenerative mouse model [29]. These results indicated that catalpol was an active ingredient which could reduce ischemic damage and enhance memory. We recently discovered that catalpol was an orthosteric agonist of GLP-1Rs that functioned similarly in humans and rats and presumably acted at the same binding site as exendin (9–39). In addition, intrathecal injection of catalpol blocked formalin-induced tonic pain and its antinociceptive effect was completely blocked by exendin (9–39) [30]. It is not known whether the neuroprotective effect of catalpol is via activation of GLP-1Rs in the brain and subsequent expression of β-endorphin.

In this study, we explored the possible involvement of the hippocampal microglial GLP-1R/β-endorphin pathway in the neuroprotective effects of both exenatide and catalpol. We first confirmed the neuroprotective effects of intracerebroventricular (i.c.v.) injection of exenatide and catalpol in a rat transient ischemia/reperfusion model. We then tested whether the GLP-1R antagonist exendin (9–39) blocked the i.c.v. injection of catalpol- and exenatide-induced neuroprotection. We further examined whether treatment with exenatide and catalpol stimulated β-endorphin expression in the hippocampus and primary cultures of microglia. We finally tested whether i.c.v. injection of the specific β-endorphin antiserum and selective opioid receptor antagonist naloxone reversed the neuroprotective effects of exenatide and catalpol. Our results suggest, for the first time, that exenatide and catalpol exert their neuroprotective effects via stimulation of the GLP-1R/β-endorphin pathway probably in hippocampal microglia.

2. Materials and methods

2.1. Animals and drugs

Male adult and 1-day old neonatal Wistar rats (250–300 g), purchased from the Shanghai Experimental Animal Institute for Biological Sciences (Shanghai, China), were maintained under temperature- and light-controlled conditions (20–24 °C), 12-h light/dark cycle) with continuous access to food and water. The rats were acclimated to the laboratory environment for 3–5 days before the study began. All of the experiments were performed in accordance with the Animal Care and Welfare Committee of the Shanghai Jiao Tong University, and followed the animal care guidelines of the National Institutes of Health.

Exenatide and exendin (9–39) were obtained from Kaijie Bio-Pharmaceutical Co. (Chengdu, China) and Shanghai TASH Biotechnology Co. (Shanghai, China). Catalpol (purity ~98% by HPLC) and naloxone hydrochloride were purchased from Biopurity Phytochemicals (Chengdu, China) and Sigma–Aldrich (Shanghai, China), respectively. The rabbit polyclonal antibodies neutralizing β-endorphin was purchased from Phoenix Pharmaceuticals (Burlingame, CA, USA). Based on the manufacturer's information, the β-endorphin antiserum was specific to β-endorphin and did not cross-react with methionine-enkephalin, leucine-enkaphalin, dynorphin A or B, γ-endorphin, α-endorphin, ACTH or α-melanocyte-stimulating hormone. All of the drugs or reagents were freshly dissolved in saline before use.

2.1.1. Immunofluorescence staining [2]

Single and double immunofluorescence labeling of the GLP-1R and microglia were performed on the hippocampi. Rats were anesthetized by pentobarbital injection (50 mg/kg) and intracardially perfused with 500 mL normal saline followed by 300 mL of 4% paraformaldehyde (wt/vol) in phosphate buffered saline. The brain of each rat was rapidly removed and the complete hippocampus was collected and fixed in 4% buffered paraformaldehyde at 4 °C for 24 h. Tissues were entrapped in OCT embedding agent (Leica Microsystems, Wetzlar, Germany) and cut into 40 μm-thick frozen sections, which were then incubated with GLP-1R antibody (ab119287, 1:100, rabbit polyclonal, Abcam, Cambridge, UK) and OX42 antibody (ab1211, 1:200, mouse polyclonal, Abcam) for microglia at 4 °C for 24 h. The GLP-1R antibody was tested for optimal dilution and nonspecific staining [2]. The GLP-1R and OX42 antibodies were visualized with Alexa 555-conjugated goat anti-rabbit secondary antibody (1:200, Invitrogen, Grand Island, NY, USA) and Alexa 488-conjugated goat anti-mouse secondary antibody (1:200, Invitrogen), respectively. DAPI (4′,6-diamidino-2-phenylindole, Sigma–Aldrich) staining was used to determine the cell nuclei.

The OX42 and GLP-1R-immunopositive cell profiles were quantified using a Leica TCS SP5II confocal microscope (Leica Microsystems). Identical confocal acquisition parameters were set for GLP-1R and OX42 staining, including objective, laser power, photomultiplier gain and offset, emission window, pinhole, and speed. Images of the CA1 and dentate gyrus regions were captured under 10× magnification. All positively stained cells in the stratum lacunosum moleculare layer of the CA1 region were measured using a computer-assisted image analysis program (Image J Software, National Institutes of Health, MD, USA) in a blinded fashion. Low and high thresholds were set to exclude background fluorescence and to include immunofluorescent intensity measurements only from positively stained cell surfaces. We determined the thresholds visually be moving the cursor in the displayed histogram until all the background pixels in the image were darkened; the intensity value at the position of the cursor was taken as the threshold value for the channel. The cell showing a two-fold more intense staining than the average background was considered positive. A colocalization analysis was performed using Image J software with a Colocalization Finder to generate images in which colocalized pixels appeared as white. The same threshold value configuration was used to measure all of the surface areas in each experimental group at the same time. The measured areas were