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NEUROPROTECTIVE AND ANTI-APOPTOTIC EFFECTS OF LIRAGLUTIDE IN THE RAT BRAIN FOLLOWING FOCAL CEREBRAL ISCHEMIA

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Abstract—Stroke is a leading cause of death and serious, long-term disability worldwide. We report that rats receiving liraglutide show markedly attenuated infarct volumes and neurological deficit following ischemic insult. We have also investigated the effect of liraglutide on apoptosis and oxidative stress pathways after ischemic injury in diabetic and non-diabetic rats. Male Sprague-Dawley rats weighing 300–350 g were used. Diabetes was induced by streptozotocin. Rats were pretreated with either vehicle or liraglutide (50 μg/kg, s.c.) for 14 days and thereafter subjected to middle cerebral artery occlusion (MCAO). Twenty-four hours after occlusion, rats were assessed for neurological deficit, motor function and subsequently sacrificed for estimation of infarct volume, oxidative stress and apoptotic markers. Vehicle-treated non-diabetic and diabetic rats showed significant ($p < 0.001$) neurological deficit following cerebral ischemia. Liraglutide pretreatment resulted in significantly ($p < 0.001$) less neurological deficit compared to vehicle-treated MCAO rats. Cerebral ischemia produced significant ($p < 0.0001$) infarction in vehicle-treated rats; however, the infarct volume was significantly ($p < 0.001$) less in liraglutide-pretreated rats. Oxidative stress markers were increased following ischemia but were attenuated in liraglutide-treated rats. Anti-apoptotic protein Bcl-2 expression was decreased and pro-apoptotic protein Bax expression was increased in vehicle-treated MCAO rats compared to sham ($p < 0.0001$). On the other hand liraglutide pretreatment showed significantly ($p < 0.01$) increased expression of Bcl-2 and decreased expression of Bax in MCAO rats. In vehicle-treated group, the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells significantly ($p < 0.0001$) increased in the ischemic hemisphere compared to sham-operated group. The number of TUNEL-positive cells in vehicle group was 73.5 ± 3.3 and 85.5 ± 5.2/750 μm^2 in non-diabetic and diabetic vehicle-treated MCAO rats, respectively. Following liraglutide treatment the number of TUNEL-positive cells was remarkably attenuated to 25.5 ± 2.8 and 41.5 ± 4.1/750 μm^2 ($p < 0.001$) in non-diabetic and diabetic rats, respectively. The results demonstrate that glucagon-like peptide 1 (GLP-1) agonist, liraglutide, is a neuroprotective agent and attenuates the neuronal damage following cerebral ischemia in rats by preventing apoptosis and decreasing oxidative stress.

Key words: cerebral ischemia, GLP-1 receptor, liraglutide, oxidative stress, apoptosis.

INTRODUCTION

Stroke is the leading cause of adult disability and represents a major health and economical threat (Donnan et al., 2008). Despite the severity of this condition, the only currently available FDA-approved pharmacological treatment for ischemic stroke is recombinant tissue plasminogen activator (rtPA), which has complications of a relatively short window of time between infarct and treatment (3–4 h) and an increased risk of subarachnoid hemorrhage (Micieli et al., 2009). A large number of other agents, broadly classified as neuroprotective and aiming to slow or stop the secondary damage associated with the ischemic cascade following stroke, have shown promise in the initial stages of research but have thus far failed to demonstrate efficacy in clinical trials (Ly et al., 2006; Stankowski and Gupta, 2011). Unfortunately, to this point, with the notable exception of rtPA, no pharmaceutical interventions have proven efficacious in human clinical trials. A new approach is therefore needed to investigate these conditions and the pathways and mechanisms which lead to them in an attempt to fully understand the etiology of the disease and where it may be curtailed by human intervention.

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular events, including stroke (Luitse et al., 2012). In addition, patients with T2DM have two- to sixfold increased risk for severe strokes and have worse outcome than patients without T2DM (Megherbi et al., 2003; Reeves et al., 2010). Moreover, it increases the risks of morbidity and mortality after stroke. Oxidative

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Abbreviations: AD, Alzheimer's disease; EDTA, ethylenediaminetetraacetic acid; Ex-4, exendin-4; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; GSH, glutathione; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; NGF, nerve growth factor; PFA, paraformaldehyde; ROS, reactive oxygen species; RPM, rotations per minute; rtPA, recombinant tissue plasminogen activator; SOD, superoxide dismutase; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TTC, 2,3,5-triphenyltetrazolium chloride; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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stress plays an essential role in the pathogenesis of cerebral ischemic injury (Vinik and Flemmer, 2002; Niizuma et al., 2009), which causes apoptosis and delayed death of cells through oxidative damage to lipids, proteins, and DNA in the ischemic penumbral region (Warner et al., 2004; Nakka et al., 2008; Niizuma et al., 2010). Assessing new stroke therapies for patients with diabetes mellitus is essential, because diabetes mellitus is an important risk factor for stroke.

Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is released into the blood stream postprandially from the gut and binds to the GLP-1 receptor (GLP-1R) (Bell et al., 1983). Currently, the GLP-1 receptor agonists exendin-4 (Ex-4), liraglutide and lixisenatide are approved for treatment of T2DM (Lovshin and Drucker, 2009; Wohlfart et al., 2013). A selective inhibitor of dipeptidylpeptidase-4 (DPP-4), functioning as a long-acting agonist of GLP-1, is also in clinical use worldwide for patients with T2DM (Drucker and Nauck, 2006; Darsalia et al., 2013; Yang et al., 2013). The beneficial effects of GLP-1 are not limited to the treatment of diabetes but also produce significant neuroprotection in animal models of cerebral ischemia and Alzheimer's disease (AD) (During et al., 2003; Lee et al., 2011; Briyal et al., 2012; Sato et al., 2013; McClean and Holscher, 2014).

GLP-1 acts as a growth factor in the brain (Buteau et al., 1999) and has been shown to protect against oxidative injury (Perry et al., 2007). Furthermore, the distribution of GLP-1R in the brain suggests they play a central role in the regulation of neuronal activity and protect the brain tissue (Banks et al., 2004; Sharma et al., 2014). GLP1-Rs have become well accepted as having anti-apoptotic properties. Both GLP-1 and Ex-4 augment cellular integrity and overall survival following exposure to a range of pro-apoptotic agents such as peroxides, cytokines and fatty acids (Hui et al., 2003; Li et al., 2003; Harkavyi and Whitton, 2010). In addition GLP-1 appears to increase expression of anti-apoptotic genes Bcl-2 and Bcl-xl (Buteau et al., 2004). Studies have also shown that Ex-4 enhances nerve growth factor (NGF)-induced neuronal differentiation and attenuates neural degeneration following NGF withdrawal indicating a potential neuroprotective role of GLP-1Rs (Drucker, 2001; Perry et al., 2002a,b).

Liraglutide is an analog of GLP-1 that acts through GLP-1R. It has been shown that liraglutide improves cognitive function and reduces amyloid plaque deposition in a mouse model of AD, and it is now being tested in clinical trials in AD patients (McClean and Holscher, 2014). Liraglutide crosses the blood–brain barrier and has neuroprotective effects in rats (Hunter and Holscher, 2012). Studies in our laboratory using the middle cerebral artery occlusion (MCAO) model of focal cerebral ischemia in rats demonstrated that chronic administration of Ex-4 significantly improved infarct volume, neurological deficit and oxidative stress parameters in ischemic rats (Briyal et al., 2012). In another study, it was found that liraglutide, administered intraperitoneally after induction of stroke, reduced infarct volume, oxidative stress parameters and increased cortical vascular endothelial growth factor (Sato et al., 2013).

Patients with T2DM may be receiving liraglutide as part of their treatment. It was thought worthwhile to investigate whether liraglutide treatment will offer any protection to the CNS damage following cerebral ischemia. In an effort to more closely model the clinical situation, we studied the effect of liraglutide given subcutaneously 2 weeks prior to the induction of ischemia stroke in diabetic and non-diabetic rats. The effects of GLP-1 receptor agonist liraglutide on infarct area, neurological and motor deficit, oxidative stress, and apoptotic markers were determined in a rat model of MCAO.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats (300–350 g) obtained from Harlan, Indianapolis, IN were allowed to acclimate for at least 4 days before use. Rats were housed in a room with controlled temperature (23 ± 1 °C), humidity (50 ± 10%), and light (6:00 A.M. to 6:00 P.M.). Food and water were available continuously. Care and use of rats along with experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Midwestern University.

Drugs

Ketamine (Butler Animal Health Supply, Dublin, OH, USA) and xylazine (Lloyd Laboratories, Shenandoah, IA, USA) were administered intraperitoneally (i.p.) in doses of 100 mg/kg and 10 mg/kg, respectively. Streptozotocin (Enzo Life Sciences, Inc., Farmingdale, NY, USA) was freshly dissolved in 0.01 M sodium citrate buffer (pH 4.3) and administered i.p. in the dose of 45 mg/kg. Liraglutide (Novo Nordisk, Inc., Princeton, New Jersey, USA) was dissolved in saline and administered at the dose of 50 μg/kg, subcutaneously (s.c.) for 14 days prior to MCAO.

Experimental protocol

Rats were randomly divided into five groups of six animals each. Group 1 animals were subjected to a sham operation. Rats in groups 2–6 underwent MCAO and were treated as follows – Group 2: Vehicle + MCAO (ND); Group 3: Liraglutide + MCAO (ND); Group 4: Vehicle + MCAO (D); Group 5: Liraglutide + MCAO (D); Group 6: Insulin + MCAO (D); (ND: Non-diabetic and D: Diabetic).

T2DM was induced in rats belonging to the diabetic group by administering streptozotocin (STZ). Rats were fed with high-fat diet (Research Diets, Inc., New Brunswick, NJ, USA) for 2 weeks prior to STZ administration. On day 3, blood glucose was obtained from the rat-tail and tested for hyperglycemia using the SureStep Complete Blood Glucose monitor kit. Rats with blood glucose value > 300 mg/dL were considered diabetic.