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ANGIOTENSIN-(1–7) ADMINISTRATION ATTENUATES ALZHEIMER'S DISEASE-LIKE NEUROPATHOLOGY IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES VIA MAS RECEPTOR ACTIVATION

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Abstract—Diabetes mellitus (DM) is associated with cognitive deficits and an increased risk of Alzheimer's disease (AD). Recently, a newly identified heptapeptide of the renin-angiotensin system (RAS), angiotensin-(1–7) [Ang-(1–7)], was found to protect against brain damage. This study investigated the effects of Ang-(1–7) on diabetes-induced cognitive deficits. Sprague–Dawley rats were randomly divided into four groups. Diabetes was induced via single *i.p.* streptozotocin (STZ) injections. Ten weeks after diabetes induction, rats in each group received an intracerebral-ventricular (ICV) infusion of either vehicle, Ang-(1–7) alone, or Ang-(1–7) + A779 daily for two weeks. At the end of the study, Morris water maze (MWM) tests were performed to test cognitive functions before the rats were euthanized. Ang-(1–7) treatment significantly reduced escape latencies in diabetic rats in acquisition trials and markedly enhanced platform area crossing frequency and time spent in the target quadrant in probe trials (3.0 ± 0.39 vs. 1.0 ± 0.33, 39.39 ± 1.11% vs. 25.62 ± 3.07%, respectively, $P < 0.01$). Ang-(1–7) treatment ameliorated damage to the ultrastructure of hippocampal synapses, reduced the expression of hippocampal phospho-tau at Ser396 ($P < 0.01$), Ser404 ($P < 0.01$) and Ser202/Thr205 ($P < 0.05$), and decreased amyloid-β oligomer and both soluble and insoluble β-amyloid peptide 1–42 (Aβ 1–42) and Aβ 1–40 levels ($P < 0.01$). These protective effects were significantly reversed by the co-administration of A779. These findings show that Ang-(1–7) is a promising therapeutic target for diabetes-induced cognitive impairment. The neuroprotective effects of Ang-(1–7) were mainly through Mas receptor (MasR) activation.

Key words: diabetes mellitus, Alzheimer's disease, angiotensin-(1–7), tau, amyloid-β.

INTRODUCTION

Diabetes mellitus (DM) is a serious metabolic disorder that affects 171 million people worldwide, and this number is expected to double by 2030 (McCrimmon et al., 2012). Previous studies have demonstrated that DM increases the risk of Alzheimer's disease (AD) (Arvanitakis et al., 2004; Biessels et al., 2006), which is the leading cause of dementia in the elderly. The pathological hallmarks of AD include widespread neuronal degeneration, extracellular accumulation of β-amyloid peptides (Aβs) into amyloid plaques and the intracellular formation of paired helical filaments (PHFs) (Huang and Mucke, 2012). Interestingly, AD-like neuropathy is also found in patients with diabetes, which ultimately leads to cognitive impairment (Dar et al., 2014). To date, the etiology and pathogenesis of aggravated AD-like neuropathy in diabetic patients are still poorly understood.

The renin-angiotensin system (RAS) is critical for the maintenance of cardiovascular function and hydroelectrolytic homeostasis in the peripheral circulation. Moreover, it has been shown that the brain has its own intrinsic RAS apart from the one in peripheral tissues (von Bohlen und Halbach and Albrecht, 2006). In the last decade, the activation of the classic RAS axis comprising angiotensin converting enzyme (ACE), angiotensin II (Ang II) and the angiotensin AT1 receptor (AT1R) has been implicated in the pathophysiology of multiple diabetes-related complications, such as diabetic cardiomyopathy and diabetic nephropathy (Masuda et al., 2012; Gagliardini et al., 2013). Similar to findings in peripheral tissues, increased levels of Ang II in the brain have been observed in diabetic rats, which may aggravate AD-like neuropathology, ultimately leading to cognitive dysfunction and dementia (Zhu et al., 2011; Mogi et al., 2012; Tian et al., 2012). Recently, a new heptapeptide of the RAS, angiotensin-(1–7) [Ang-(1–7)], which acts to counterbalance most of the effects of Ang II has aroused considerable attention. Ang-(1–7) is an

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Abbreviations: ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; Ang-(1–7), angiotensin-(1–7); AT1R, AT1 receptor; Aβ, β-amyloid peptide; DM, diabetes mellitus; GSK-3β, glycogen synthase kinase-3β; ICV, intracerebral-ventricular; IHC, immunohistochemistry; LTP, long-term potential; MWM, Morris water maze; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PHFs, paired helical filaments; RAS, renin-angiotensin system; STZ, streptozotocin; TEM, transmission electron microscope.

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endogenous peptide formed from Ang II by angiotensin converting enzyme-2 (ACE2), which acts primarily through binding to a G-protein-coupled receptor called the MAS receptor (MasR) (Donoghue et al., 2000; Santos et al., 2003). The effect of Ang-(1–7) can be blocked by A779 [D-Ala7-Ang-(1–7)], a specific Mas antagonist (Santos et al., 1994). Accumulating evidence suggests that supplementation with exogenous Ang-(1–7) or increased levels of endogenous Ang-(1–7) could reduce the risk of developing diabetic cardiomyopathy, nephropathy, retinopathy, angiopathy, and insulin resistance, as well as other associated diseases (Verma et al., 2012; Yousif et al., 2012; Jarajapu et al., 2013; Cao et al., 2014; Mori et al., 2014; Papinska et al., 2015; Shi et al., 2015). More recently, studies have surfaced demonstrating the neuroprotective effects of Ang-(1–7) in various animal models, such as those of hypertensive encephalopathy, stroke and chronic cerebral hypoperfusion (Jiang et al., 2013; Chen et al., 2014; Xie et al., 2014; Jiang et al., 2014b). Moreover, Ang-(1–7) in the brain has been shown to play an important role in cognitive processes (Gironacci et al., 2013). A recent study indicated that Ang-(1–7)/Mas axis integrity is essential for normal object recognition memory processing (Lazaroni et al., 2012). There is also emerging evidence that Ang-(1–7) levels are reduced in animal models of AD during disease progression. Furthermore, an inverse correlation was found between Ang-(1–7) levels and tau hyperphosphorylation, suggesting that Ang-(1–7) may participate in the pathogenesis of AD (Jiang et al., 2015).

However, the role of the ACE2/Ang-(1–7)/Mas axis in the cognitive function of DM patients remains unknown. Based on previous findings of the neuroprotective effects of Ang-(1–7), we hypothesized that Ang-(1–7) may alleviate diabetes-induced cognitive impairment via the modulation of MasR. In the current study, we investigated the potential roles of Ang-(1–7) in rats with streptozotocin (STZ)-induced diabetes and AD-like functional and pathological changes in their brains, and we provide new insights into the possible neuroprotective mechanisms of the ACE2/Ang-(1–7)/Mas axis associated with DM.

EXPERIMENTAL PROCEDURES

Animals

Six-week-old male Sprague–Dawley rats (weight, 180–210 g) were purchased from the animal center of Chongqing Medical University. All animals were housed five per cage in a temperature-controlled room (22 ± 2 °C) with a 12 h light/dark cycle (lights on 8 a.m.–8 p.m.) and were given free access to food and water. All experimental procedures were carried out during the light phase. The animal protocol was approved by the Animal Care and Use Committee of The First Affiliated Hospital of Chongqing Medical University. All animal experiments followed the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal studies complied with the ARRIVE guidelines.

Experimental protocols

The rats (n = 60) were randomly selected and allocated to four groups: (1) control group; (2) diabetic group (DM group); (3) DM + Ang-(1–7) treatment group [Ang-(1–7) group]; or (4) DM + Ang-(1–7) + A779 treatment group [Ang-(1–7) + A779 group]. STZ, Ang-(1–7) and A779, which is a specific MasR antagonist, were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). After a one-week acclimation period, diabetes was induced via a single *i.p.* injection of STZ (55 mg/kg body weight) dissolved in a 0.1 M sodium citrate buffer (pH 4.4). The control group received an *i.p.* injection of an equivalent volume of citrate buffer. Diabetes was confirmed based on fasting blood glucose levels over 16.67 mmol/L after three days of STZ injections. Random blood glucose levels were measured in blood collected from the tail vein at 4, 8 and 12 weeks after the induction of diabetes. Ang-(1–7) and A779 were dissolved in an artificial cerebrospinal fluid (aCSF, pH 7.4, composition in mM: NaCl 130, KCl 2.99, $CaCl_2$ 0.98, $MgCl_2$ 0.80, $NaHCO_3$ 25, NaH_2PO_4 0.46, Na_2HPO_4 0.039). Intracerebral-ventricular (ICV) cannulations were performed as previously reported (Ma et al., 2011). Ten weeks after the induction of diabetes, the DM + Ang-(1–7) group and DM + Ang-(1–7) + A779 group were infused with Ang-(1–7) (2.5 nmol, 2 μl) and Ang-(1–7) (5 nmol, 1 μl) + A779 (50 nmol, 1 μl), respectively, into the right lateral cerebral ventricular once per day for 14 consecutive days. The control and DM groups received an equivalent volume of aCSF. The doses of Ang-(1–7) and A779 used were based on our previous work (Zhang et al., 2015).

Morris water maze

Cognitive performance was evaluated for six consecutive days using a Morris water maze (MWM) after two weeks of ICV infusions. The water maze apparatus (130 cm diameter, 50 cm height) was divided into four quadrants and filled with water at 22 ± 1 °C to a depth of 30 cm. The escape platform (10 cm diameter) was located in a designated target quadrant in a permanent position 1–2 cm below the surface of the water. The rats were given four training trials per day for five consecutive days. For each trial, the rat was placed in the pool (facing the pool wall) at one of the selected quadrants. Each trial lasted until the rat found the platform or until a maximum of 90 s had elapsed. If the rat failed to find the platform within 90 s, it was guided to the platform by a technician for 15 s. Once a rat mounted the platform, it was allowed to remain there for 10 s. Acquisition was measured as escape latency to reach the platform. Twenty-four hours after the last trial, the rats were subjected to probe trials in which the platform was removed and they were allowed to swim freely for 90 s. The frequency of an individual rat passing the platform area and the time the animal spent in the target quadrant were recorded as a measure of spatial memory.

After probe trials, all rats performed a visible platform test to detect possible deficits in visual acuity and motor ability. The platform was fixed in a new quadrant 1 cm