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Angiotensin II induces interleukin-6 expression in astrocytes: Role of reactive oxygen species and NF-κB

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ABSTRACT

Previously, we showed that the bio-peptide angiotensin (Ang) II induces interleukin-6 (IL-6) in cultured astrocytes; however, the mechanism(s) involved in this effect were unknown. In the current study, we determined in brainstem and cerebellum astrocytes from the spontaneously hypertensive rat (SHR), the effect of Ang II to induce IL-6 as well as reactive oxygen species (ROS) generation. Results from this study showed that Ang II significantly induced the differential expression of IL-6 mRNA and protein levels in astrocytes from both regions of Wistar and SHRs. There were differences in the ability of Ang II to induce IL-6 mRNA and protein levels, but these differences were not apparent at all time points examined. Ang II also induced ROS generation, but there were no significant differences between ROS generation in SHR samples as compared to the Wistar samples. Ang II-induced IL-6 levels were mediated via the AT_1/Nuclear Factor Kappa beta/ROS pathway. Overall, our findings suggest that there may be dysregulation in IL-6 production from astrocytes, contributing to differences observed in SHRs versus its normotensive control. Elucidating the mechanisms involved in Ang II pro-inflammatory effects in the central nervous system may lead to the development of novel therapeutic strategies that can be harnessed not just to treat hypertension, but other Ang II-mediated diseases as well.

1. Introduction

The pathophysiological mechanisms underlying regulation of blood pressure are complex; however, it is well recognized that the peptides produced by the renin-angiotensin system (RAS) greatly contribute to maintenance of blood pressure (Mehta and Griendling, 2007). Ang II is the most well-known peptide produced by this system. This peptide is a potent biologically active octapeptide with multiple activities on host cells and tissues. Ang II has emerged as a critical hormone that affects the functions of virtually all organs including the heart, kidneys, the vasculature, and the brain. Given its diverse functions and its potency in affecting cardiovascular physiology, it is not surprising that dysregulation of Ang II effects is tied to numerous cardiovascular diseases including hypertension, congestive heart failure, and others (Mehta and Griendling, 2007; Veerasingham and Raizada, 2003). Indeed, agents that target the actions of Ang II receptors (Ang receptor blockers, example Losartan), and agents that target the synthesis of Ang II (ACE inhibitors, example Lisinopril, and renin inhibitors, example Aliskiren) are popular blood pressure lowering medications.

Ang II exerts most of its actions through Ang type 1 (AT_1) receptors, and this receptor mediates effects of Ang II including systemic vasoconstriction and cell proliferation (Mehta and Griendling, 2007; Murphy et al., 1991; Mukoyama et al., 1993; Kambayashi et al., 1993). It is this receptor that is blocked by Ang receptor blockers such as Losartan. Alternatively, Ang II can also act on AT_2 receptors to cause vasodilation and apoptosis (Griendling et al., 1997; Tallant et al., 1991; Hunyady and Catt, 2006). Hyperactivity of the brain RAS has been implicated in the development of

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International; ACE, Angiotensin Converting Enzyme; Ang, Angiotensin; AT1R, Ang type 1 receptor; BCA, Bicinchoninic acid; DMEM/F12, Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12; FBS, Fetal Bovine Serum; GFAP, Glial Fibrillary Acidic Protein; H_2O_2, Hydrogen Peroxide; NaCl, Sodium chloride; NaF, Sodium fluoride; NF-κB, Nuclear Factor Kappa beta; $NaVO_4$, Sodium orthovanadate; PMSF, Phenylmethylsulfonyl fluoride; qPCR, quantitative PCR; RAS, Renin Angiotensin System; SHR, spontaneously hypertensive rat.

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hypertension in several types of experimental and genetic hypertension animal models. In addition, a substantial proportion of Ang II's other cardiovascular actions, e.g. heart failure, results from Ang II actions in the brain (Morimoto and Sigmund, 2002) (Matsukawa et al., 1991). Thus, it is essential to understand how Ang II works in the brain, and determine central Ang II signaling pathways that are dysregulated leading to hypertension. Identification of such signaling processes is essential for understanding the mechanisms that regulate physiological activities elicited by Ang II. These intracellular pathways may malfunction, leading to pathological consequences such as hypertension, and thus present targets for manipulation for disease prevention.

Reactive Oxygen Species (ROS) consist of a number of substances that under normal physiological conditions play a pivotal role in innate immunity, cell signaling, and regulation of vascular integrity (Bayir, 2005). These molecules consist of biologically active oxygen species/radicals namely, hydrogen peroxide (H_2O_2), hypochlorus acid, fatty acid hydroperoxidase, reactive aldehydes and singlet oxygen which are generated at low levels in a constant fashion as byproducts of normal cellular metabolism (Bayir, 2005). H_2O_2 is considered to be the most important ROS that has clinical significance, and is the key molecule induced by Ang II (Touyz, 2004). Imbalances between the production and clearance rates of ROS lead to oxidative stress, resulting in several pathophysiological processes in humans. ROS have been linked to, neurodegenerative diseases, cancer, inflammatory conditions, and diabetic mellitus (Valko et al., 2007; Sayre et al., 2001). Supporting data from many studies conducted in different cell types, such as vascular smooth muscle cells, endothelial cells, adventitial cells, and mesangial cells also suggest that Ang II induces ROS (Dhalla et al., 2000; Pagano et al., 1997; Jaimes et al., 1998; Touyz and Schiffrin, 1999; Harrison, 1997) leading to hypertension (Rajagopalan et al., 1996; Laursen et al., 1997; Touyz and Schiffrin, 2000). In the brain, ROS are best known for their role in the pathogenesis of primary neurodegenerative diseases, such as amyotrophic lateral sclerosis (Deng et al., 1993) and Alzheimer's disease (Smith et al., 1997). It has been postulated that neuronal death in these diseases may be mediated by oxidative stress caused by the aberrant metabolism of superoxides (Schulz et al., 1995).

Despite their well-known role in neurodegeneration, very little is understood about ROS as second messengers in normal neural processes in the brain, and even less is known about the role of redox mechanisms in CNS-mediated regulation of cardiovascular functions. Findings from Petterson et al. (Peterson et al., 2006) showed that excessive production of ROS in the brain plays a crucial role in the pathogenesis of Ang II-dependent hypertension. Furthermore, excessive brain ROS production has been found in various animal models of hypertension (Zimmerman and Davisson, 2004). In spontaneously hypertensive rats (SHRs) it has been shown that ROS may contribute to maintaining hypertension in these animals (Schnackenberg et al., 1998; Schnackenberg and Wilcox, 1999; Suzuki et al., 1998; Suzuki et al., 1995; Yoshioka et al., 1985; Nakazono et al., 1991). However, the data supporting the role of ROS in this hypertension model are derived from peripheral cell systems including mesenteric arteries and the kidneys (Schnackenberg et al., 1998; Tschudi et al., 1996). Given the evidence for superoxide-generating and scavenging systems throughout the brain (Lindenau et al., 2000; Shimohama et al., 2000) and the importance of ROS signaling in a wide range of Ang II-regulated cellular processes, one of the objectives of this study was to determine the role of Ang II mediated ROS generation in brain astrocytes isolated from SHRs.

Interleukin-6 (IL-6) is a multifunctional cytokine known to mediate inflammatory processes in the body (Akira and Kishimoto, 1992; Loppnow and Libby, 1990). Ang II and ROS induce IL-6 protein secretion from various cell types (Beasley, 1997). It has been shown in retinal pigment epithelial cells that Ang II increases the activities of intracellular pathways such as, ERK1/2 Extracellular signal regulated kinase mitogen activated protein (MAP) kinase, the Janus MAP kinase, and Nuclear Factor Kappa beta (NF-κB) leading to an increase in ROS and IL-6 production (Wu et al., 2010). NF-κB is a transcription factor that controls DNA transcription, cell survival and cytokine production (Gilmore, 2006; Brasier, 2006). The intracellular signaling pathways by which Ang II induces ROS and IL-6 production in astrocytes are unknown. However, previous studies suggest that both Ang II and ROS may utilize similar pathways to induce IL-6 levels. In IL-6 knockout mice, Ang II-mediated hypertension decreased, suggesting a role for this cytokine and neuroinflammatory processes generated by the cytokines in this type of hypertension (Brands et al., 2010). Previously, we have shown that Ang II induces IL-6 mRNA and protein levels from astrocytes isolated from normotensive rats but the role of ROS, NF-κB and other pathways in this effect was not tested (Kandalam and Clark, 2010; Kandalam et al., 2012). Further, whether Ang II mediates ROS and IL-6 production in astrocytes isolated from a hypertensive rat model, the SHR, is unknown and is a focus of the current study.

In this study, we used brainstem and cerebellum astrocytes isolated from Wistar and SHRs to determine whether ROS and IL-6 levels are dysregulated in the SHR and established a link between Ang II-mediated ROS generation and IL-6 production. The interrelationship between Ang II, ROS, and IL-6 production through the NF-κB signaling pathway was also determined using SHR as a genetic hypertensive animal model. The SHR was selected for these studies as it is a widely used and accepted animal model to study hypertension due to similarities exhibited in these animals as compared to humans (Okamoto and Aoki, 1963). Ang II plays a crucial role in the development and establishment of the hypertensive state in this rat (Harrap et al., 1990; Wu and Berecek, 1993). Most importantly, centrally-produced Ang II mediates hypertension in this animal (Greenwood et al., 1963). Further, astrocytes perform immune functions (Alarcon et al., 2005; Constantinescu et al., 2005; Fontana et al., 1984; Hull et al., 2006), synthesize and release neurotrophic factors and are involved in the formation of neural scars following injury (Fawcett and Asher, 1999; Silver and Miller, 2004). Thus, the findings from this study are essential in validating the immunomodulatory effects of astrocytes as these cells secrete IL-6, a function that may be different in astrocytes isolated from a hypertensive model as compared to the normotensive control. Brainstem and cerebellum areas of the brain were selected as astrocytes isolated from these regions are known to contain RAS components (Tallant and Higson, 1997). Most importantly, the brainstem is known to be involved in cardiovascular regulation. Cerebellum astrocytes were also included in these studies as astrocytes from this area of the brain was shown to express RAS components albeit at low levels (Tallant and Higson, 1997). In our laboratory, we routinely study the effects of Ang II and other peptides in both brainstem and cerebellum astrocytes (Clark and Gonzalez, 2007a,b; Clark et al., 2008).

2. Materials and methods

2.1. Reagents and test substances

Ang II was obtained from Bachem (Torrance, CA). PD123319, the selective AT_2 receptor blocker was obtained from Sigma (St. Louis, MO), and Losartan (the AT_1 receptor blocker) was kindly provided by Du Pont Merck (Wilmington, DE). The NF-κB inhibitor BAY11-7082 was purchased from Santa Cruz Biotechnology (Dallas, Texas), and YCG063 (ROS inhibitor) was bought from EMD Millipore