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Research Report

Regulation of angiotensinogen expression by angiotensin II in spontaneously hypertensive rat primary astrocyte cultures

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ABSTRACT

Background: Angiotensin (Ang) II, a bio-peptide of the renin-angiotensin system (RAS), plays a pivotal role in biological systems. It has been well established that in the brain, astrocytes are the predominant source for angiotensinogen (AGT), which is the precursor molecule for Ang II. The primary objective of this study was to determine the effect of Ang II on AGT mRNA and protein expression levels in primary cultures of astrocytes isolated from the brainstem and cerebellum regions of spontaneously hypertensive rats (SHRs) and normotensive Wistar rats.

Methods: Astrocytes were treated with 100 nM Ang II and the effect of time and the receptors involved in AGT mRNA and protein expression were measured using qPCR, and western blotting techniques, respectively.

Results: Ang II significantly downregulated AGT mRNA levels and upregulated AGT protein levels in both SHR and Wistar rat astrocytes. Basal AGT mRNA levels in SHR samples were significantly lower as compared to Wistar astrocytes isolated from brainstem and cerebellum. There was no difference in the basal AGT protein levels when SHR and Wistar samples were compared. There was a tendency for higher Ang II-induced AGT protein levels in SHR samples compared to normotensive controls, but the difference was not significant. Ang II tended to decrease AGT mRNA levels of Wistar samples to a greater degree than SHR samples. The Ang AT_1 receptor mediated the actions of Ang II on AGT protein and mRNA levels.

Conclusion: These findings highlight the complexity of AGT regulation and show that AGT protein and mRNA levels are responsive to Ang II in both SHR and Wistar astrocytes. Most importantly, our findings suggest that this peptide can induce its own synthesis by positively regulating AGT protein synthesis, an effect that was more robust in SHR astrocytes. Thus, dysregulation of this system may be important in preserving the hypertensive phenotype in this model.

1. Introduction

Despite the discovery of components of the RAS dating back more than 100 years ago (Balakumar and Jagadeesh, 2014), elucidating the components of this system and its active physiological peptides are still considered to be of great importance in understanding the pathophysiology of various diseases. It is well recognized that the peripheral and central renin-angiotensin systems (RAS) are key biochemical pathways contributing to the regulation of blood pressure (Mehta and Griendling, 2007). Angiotensin (Ang) II, the most well-known peptide produced by the RAS (Herichova and Szantoova, 2013), is derived from the precursor molecule Angiotensinogen (AGT), through the actions of the enzyme renin. This peptide has pervasive actions to control numerous physiological processes due to its effects on many hormonal and neuro-hormonal systems in the body (Atlas, 2007; Herichova and Szantoova, 2013) including the cardiovascular and inflammatory systems. Thus, dysregulation of Ang II synthesis and effects may lead to several disorders, including hypertension.

Ang II exerts its action through two pharmacological classes of G protein-coupled receptors, known as the Ang type 1 (AT_1) and type 2 (AT_2) receptors (Balakumar and Jagadeesh, 2014). In astrocytes, Ang II interaction with AT_1 receptors causes activation of several intracellular signaling pathways involving mitogen activated protein (MAP) kinases, tyrosine kinases, protein kinase C (PKC), immediate early response genes, and others (Karnik et al., 2015; Tallant and Higson, 1997). These intracellular pathways are involved in widely diverse effects of Ang II including cellular growth, proliferation, and inflammation. Ang II interactions with AT_2 receptors lead to anti-proliferative and proapoptotic changes, effects that are opposite those of the AT_1 receptors (Horiuchi et al., 1999). AT_2 receptors are

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also important in fetal development, and can be induced later in adult life under pathological conditions (Shanmugam et al., 1996).

Among the components of the RAS, AGT is a unique substrate of renin in vivo. Expression of the AGT gene is regulated in a cell type-specific and differentiation-linked manner in cell culture systems (Brasier et al., 1989; Eggena and Barrett, 1992; Fukamizu et al., 1990; McGehee et al., 1993), and the 750-bp promoter element from the immediate 5^1-flanking region is capable of directing most, but not all, tissue-specific and hormonal regulation of the AGT minigene in transgenic mice (Clouston et al., 1989). Molecular variants of the human AGT gene are associated with essential hypertension (Caulfield et al., 1994; Jeunemaitre et al., 1992), and recent studies have shown that several mutations of the proximal promoter region of the human AGT gene that affect the binding activity of transcription factors are associated with essential hypertension (Inoue et al., 1997; Ishigami et al., 1997; Sato et al., 1997). The findings of these studies suggest that transcriptional regulation of the AGT gene may be intimately involved in the pathogenesis of hypertension.

AGT is synthesized in most regions of the brain, with its most abundant production in the medulla and hypothalamus, areas with demonstrated control of cardiovascular function (Stornetta et al., 1988). Studies from Lynch et al. and others (Harrap, 1986; Lynch et al., 1986; Lynch et al., 1987; Stornetta et al., 1988) demonstrated that AGT mRNA accumulates throughout the rat brain at a low level and, in addition, several dozen nuclei in the hypothalamus, midbrain, and brainstem contain high levels of this mRNA. The most abundant source of AGT in the brain is in astrocytes; these cells have been reported to constitutively secrete AGT into the extracellular and cerebrospinal fluids (Genain et al., 1984; Ruiz et al., 1983; Stornetta et al., 1988). In addition to the AGT-positive astrocytes that are found throughout the brain, AGT is also present in a limited population of neurons in regions of the brain dedicated to cardiovascular control (Tallant and Higson, 1997). Although the relative importance of glial and neuronal AGT remains poorly defined, the importance of glial cells as a source of AGT participating in arterial blood pressure regulation is becoming better appreciated. Transgenic mice exhibiting glial-specific overexpression of human AGT (hAGT) and human renin have a moderate increase in blood pressure (Morimoto et al., 2002). Transgenic rats carrying a glial-targeted anti-sense construct to AGT exhibited a 90% decrease in brain AGT levels and a significant reduction in blood pressure (Schinke et al., 1999). Astrocyte AGT has also been reported as important for the maintenance of the blood-brain barrier in response to cold-induced brain injury (Kakinuma et al., 1998).

Hyperactivity of the brain RAS has been implicated in the development and maintenance of hypertension in several types of experimental and genetic hypertension animal models (Reaux-Le Goazigo et al., 2005). The spontaneously hypertensive rat (SHR) is a well-established model of hypertension (Okamoto et al., 1966) with a distinct cardiovascular and behavioral phenotype that makes it a useful model for the study of hypertension. As compared with controlled rats, SHRs have increased brain AGT (Naruse et al., 1985; Printz and Healy, 1983); Ang II (Phillips and Kimura, 1988); and Ang II binding capacity (Gutkind et al., 1988; Salaymeh et al., 1986). This heightened level of the RAS components in the brain has been implicated as a possible source of hypertension (Greenwood et al., 1963; Phillips, 1987), and elevated salt appetite (DiNicolantonio et al., 1982) in this model. Interruption of this system's activity by pharmacological means results in lowering of blood pressure and control of hypertension. For example, early treatment of young SHRs with angiotensin converting enzyme inhibitors (ACEIs) prevents the hypertensive state in these animals even after the treatment was stopped (Harrap et al., 1990; Wu and Berecek, 1993). It has been suggested that the RAS in SHR brains shows higher activity compared with their controls (Greenwood et al., 1963). More importantly, when an AT_1 receptor blocker or a renin inhibitor was infused into the brain of SHRs, the increased blood pressure of these animals were attenuated. In contrast, the receptor blocker had no effect on the control animals (Phillips et al., 1977). These findings suggest that hypersensitivity of central Ang II receptors is present in SHR, and that binding of Ang II to its receptor may be a component of hypertension in these animals.

Despite the important physiological roles of the brain RAS, surprisingly little is known about the mechanisms regulating AGT expression in the brain in physiological or pathological conditions. In hepatocytes, hormones such as estrogen, glucocorticoids and thyroid hormone modulate AGT levels via interactions with specific response elements located upstream of the AGT promoter region that increase promoter activity and transcription (Brasier et al., 1990; Brasier and Li, 1996). Cytokines, such as interleukin-6, alter expression of transcription factors such as the nuclear factor-kappa β complex to modulate hepatic AGT production by an interaction with the acute-phase response element (Ohtani et al., 1992; Ruiz et al., 1987; Acres et al., 2011; Brasier and Li, 1996). However, such mechanisms appear to be tissue-specific, as Ang II up-regulates AGT expression in both cultured hepatocytes and cardiac myocytes (Klett and Hackenthal, 1987; Klett et al., 1988; Malhotra et al., 1999), but decreases expression in cardiac fibroblasts (Dostal et al., 2000). These reported observations highlight that our understanding of the regulation of AGT expression is incomplete and that there are tissue- and cell-specific mechanisms by which Ang II regulates AGT expression. Thus, the aims of these studies were to examine how Ang II regulates the expression of AGT in primary cultures of astrocytes isolated from brainstem and cerebellum regions, and also to determine whether this AGT expression was dysregulated in the hypertensive state. Astrocytes were isolated from the brainstem and the cerebellum regions as they represent areas of the brain involved in cardiovascular regulation, and an area that is not involved in cardiovascular regulation, respectively. Astrocytes isolated from both regions have been shown to contain RAS components (Tallant and Higson, 1997).

2. Results

In this study, we determined whether astrocytes isolated from the SHR are responsive to Ang II and determined whether Ang II had a different effect on AGT mRNA and protein levels in SHR astrocytes as compared to astrocytes from the normotensive controls.

2.1. Effect of time on Ang II-mediated AGT mRNA expression

In order to determine the time-dependent effect of Ang II on AGT mRNA expression, subconfluent quiescent brainstem and cerebellum astrocytes from both Wistar and SHR were treated with 100 nM Ang II for various periods of time. Cells were harvested at 4h, 8h, 12h, 16h and 24h after Ang II treatment. Cells that were not treated with Ang II were used as the controls. AGT mRNA expression was measured as described. Compared to basal levels, Ang II treatment decreased AGT mRNA expression in both SHR and Wistar brainstem and cerebellum samples (Figs. 1 and 2). Further, the extent of inhibition showed differences when results from SHR and Wistar samples were compared. In brainstem as well as cerebellum astrocytes [Figs. 1 and 2], treatment with Ang II led to a more pronounced decrease in overall mRNA levels in astrocytes isolated from Wistar as compared to SHR astrocytes. In cerebellum samples [Fig. 2] this difference was statistically significant at the 12 h Ang II exposure time point when Wistar and SHR samples were compared. No significant differences were observed when brainstem Wistar and SHR mRNA samples were compared [Fig. 1]. In addition, direct comparison of the basal cycle threshold (Ct) values for Wistar and SHR AGT mRNA expression in