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ABERRANT EXPRESSION OF THE PORE-FORMING K_{ATP} CHANNEL SUBUNIT Kir6.2 IN HIPPOCAMPAL REACTIVE ASTROCYTES IN THE 3xTg-AD MOUSE MODEL AND HUMAN ALZHEIMER'S DISEASE

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Abstract—Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by beta-amyloid (Aβ) deposition, neurofibrillary tangles and cognitive decline. Recent pharmacologic studies have found that ATP-sensitive potassium (K_{ATP}) channels may play a role in AD and could be a potential therapeutic target. Interestingly, these channels are found in both neurons and astrocytes. One of the hallmarks associated with AD is reactive gliosis and a change in astrocytic function has been identified in several neuropathological conditions including AD. Thus the goal of this study was to examine whether the pore-forming subunits of K_{ATP} channels, Kir6.1 and Kir6.2, are altered in the hippocampus in a cell type-specific manner of the 3xTg-AD mouse model of AD and in human AD tissue obtained from the Chinese brain bank. Specifically, in old 3xTg-AD mice, and age-matched controls, we examined glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), Kir6.1 and Kir6.2 in hippocampal region CA1 with a combination of immunoblotting and immunohistochemistry (IHC). A time point was selected when memory impairment and histopathological changes have been reported to occur in 3xTg-AD mice. In human AD and age-matched control tissue IHC experiments were performed using GFAP and Kir6.2. In the hippocampus of 3xTg-AD mice, compared to wild-type controls, Western blots showed a significant increase in GFAP indicating astrogliosis. Further, there was an increase in Kir6.2, but not Kir6.1 in the plasma membrane fraction. IHC examination of hippocampal region CA1 in 3xTg-AD sections revealed an increase in Kir6.2 immunoreactivity (IR) in astrocytes as identified by GFAP and GS. In human AD tissue similar data were obtained. There was an increase in GFAP-IR in the stratum oriens (SO) and alveus (ALV) of CA1 concomitant with an increase in Kir6.2-IR in cells with an astrocytic-like morphology. Dual immunofluorescence revealed a dramatic increase in co-localization of Kir6.2-IR and GFAP-IR. Taken together, these data demonstrate that increased Kir6.2 is seen in reactive astrocytes in old 3xTg-AD mice and human AD tissue. These changes could dramatically alter astrocytic function and subsequently contribute to AD phenotype in either a compensatory or pathophysiological manner.

Key words: astrogliosis, CA1, GFAP, inwardly rectifying potassium channels, Kir6.1, glutamine synthetase.

INTRODUCTION

Astrocytes are the most common cell type found in the central nervous system (CNS). They are a heterogeneous population of cells whose morphology and function can vary depending on location, subtype and developmental stage. Based on their cytoarchitecture and other physiological characteristics they are ideally suited to sense and respond to changes in their local surroundings. Astrocytes are known to participate in the regulation of a variety of critical CNS functions including: (1) neurometabolism (Tsacopoulos and Magistretti, 1996), (2) ionic and osmotic homeostasis (Simard and Nedergaard, 2004), (3) energy storage (Cataldo and Broadwell, 1986; Magistretti et al., 1993; Brown and Ransom, 2007), (4) the formation, activity and remodeling of synapses (Stevens, 2008; Perea et al., 2009), (5) the release of gliotransmitters (Araque et al., 2014), (6) regulation of the blood–brain barrier and blood flow (Abbott et al., 2006) and (7) the defense against oxidative stress (Bélanger et al., 2011). Further, astrocytes respond to CNS insults such as infection, trauma and neurodegenerative disease by undergoing

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Abbreviations: AD, Alzheimer's disease; ALV, alveus; Aβ, beta amyloid; CNS, central nervous system; DAB, diaminobenzidine; GAPDH, glyceraldehyde-3-phosphate; GFAP, glial fibrillary protein; GS, glutamine synthetase; HH3, phosphorylated histone H3; IHC, immunohistochemistry; IR, immunoreactivity; Kir, inwardly rectifying potassium channel; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; SUR, sulfonylurea.

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reactive astrogliosis, a process that entails dramatic changes in gene expression and morphology (Sofroniew, 2009; Sofroniew and Vinters, 2010). Dysfunction of astrocytes has recently been identified in numerous neuropathological diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis, depression, Huntington's disease, ischemia/stroke, and Parkinson's disease (Sofroniew and Vinters, 2010; Pekny et al., 2015).

AD is a progressive neuropathological condition characterized by a decline in learning, memory and cognitive function that eventually results in dementia. Since its description more than 100 years ago, tremendous insight has been gained into the factors associated with AD including: (1) the formation of beta amyloid (Aβ) plaques and neurofibrillary tangles (Hardy et al., 1998), (2) astrogliosis (Chen et al., 2014; Osborn et al., 2016; Rodríguez et al., 2009a,b), (3) a decline in cholinergic innervation (Whitehouse et al., 1981; Arendt et al., 1983; Arendt and Bigl, 1986; German et al., 2003), (4) inflammation (Hüll et al., 1996), (5) oxidative stress (Good et al., 1996), (6) genetic predisposition (Goate et al., 1991; Tsai et al., 1994; Sherrington et al., 1995), (7) synapse and/or cell loss (Ball, 1977), and (8) regional CNS hypometabolism (Kennedy et al., 1995; Reiman et al., 1996; Mosconi et al., 2008). Despite these advances, AD is the 6th leading cause of death in America and currently there is no therapeutic strategy to prevent, cure or slow its progression. Therapeutic strategies have primarily targeted the decline in cholinergic innervation or the formation of beta amyloid plaques and though recent studies have provided evidence that Aβ-antibody-based vaccinations may have promise (Frost et al., 2015; Wisniewski and Goñi, 2015), there is still a tremendous need for identifying new potential therapeutic targets.

ATP-sensitive potassium (K_{ATP}) channels may be such a target. Recent studies have demonstrated that: (1) the toxic form of β-amyloid (β-amyloid$_{1–42}$) can increase the expression of K_{ATP} channel subunits in primary neuronal cell culture (Ma et al., 2009), (2) chronic treatment of the 3xTg-AD mouse model of AD with diazoxide, a K_{ATP} channel activator, can reduce plaque and tau pathology and somewhat preserve memory relative to vehicle-treated 3xTg-AD controls (Liu et al., 2010), and (3) the direct administration of the K_{ATP} channel blocker glibenclamide into the hippocampus of APP/PS1 mice (another mouse model of AD) can increase interstitial β-amyloid levels, while K_{ATP} channel openers (diazoxide and pinacidil) can block the increase in interstitial β-amyloid triggered in APP/PS1 mice by a hyperglycemic challenge (Macauley et al., 2015). These studies demonstrate that K_{ATP} channel expression can be altered by β-amyloid and that K_{ATP} channels can also affect AD pathogenesis/phenotype. While the prior studies using pharmacological manipulation of K_{ATP} channels attributed their results to modulation of neuronal activity it is highly plausible that astrocytes that may be involved as K_{ATP} channels are also present in astrocytes (Thomzig et al., 2001, 2005; Zhou et al., 1999, 2002, 2012). Further, studies of other neuropathologies that are associated with reactive gliosis such as traumatic brain injury (D'Ambrosio et al., 1999), temporal lobe epilepsy (Bordey and Spender, 2004) and entorhinal cortex lesions (Schröder et al., 1999) have reported alterations (decrease) in inwardly rectifying potassium currents in astrocytes. However, no data are currently available regarding the protein levels and distribution of K_{ATP} channels in neurons or astrocytes in AD.

In excitable cells, K_{ATP} channels are generally thought to couple cellular electrical activity with cellular metabolism. Specifically, K_{ATP} channel activity is dependent on the intracellular ATP/ADP ratio with an increase in ATP levels causing channel closure and consequently membrane depolarization while a decrease in intracellular ATP causes channel opening and cell hyperpolarization. However, the total functional significance of K_{ATP} channels in the central nervous system is unclear. Emerging data suggest that CNS K_{ATP} channels may also be involved in: (1) neuroprotection (Yamada and Inagaki, 2005), (2) neurotransmitter release via presynaptic mechanisms (Amoroso et al., 1990; Tanaka et al., 1995), and (3) astrocytic coupling via gap junctions which subsequently can affect the capacity of astrocytes to regulate extracellular K^+ and glutamate (Newman et al., 1984; Velasco et al., 2000; Danbolt, 2001; Sun et al., 2008).

K_{ATP} channels are heterooctameric complexes composed of four Kir subunits (Kir6.1 or Kir6.2) that create the channel's pore region and four regulatory sulfonylurea subunits (SUR1, SUR2A, SUR2B) (Aguilar-Bryan et al., 1998; Babenko et al., 1998; Ashcroft and Gribble, 2000). Regarding the two main pore-forming subunits, anatomical data suggest that in the hippocampus Kir6.1 is preferentially found in astrocytes and the inner mitochondrial membrane of neurons, although Kir6.1 has also been detected in the plasma membrane of neurons (Zhou et al., 1999, 2002; Zawar et al., 1999; Thomzig et al., 2001). In other structures however, (dorsal root ganglia, dorsal vagal neurons, retina) data have suggested that Kir6.1 is primarily restricted to glia cells (Zawar et al., 1999; Eaton et al., 2002; Skatchkov et al., 2002; Zoga et al., 2010). In comparison, Kir6.2 has been suggested to be primarily localized in neurons (Thomzig et al., 2005) with the expression of Kir6.2 in glial cells being a subject of controversy. For example, using whole-cell patch clamp recordings McKhann et al. (1997), found that astroglial cells do not express K_{ATP} channels and Dunn-Meynell et al. (1998) reported that while various neurons express Kir6.2 mRNA neither astrocytes nor oligodendrocytes express Kir6.2 mRNA. However, using patch clamp recordings Zawar et al. (1999) showed that both neurons and glial cells in the hippocampus were sensitive to K_{ATP} channel openers and inhibitors and Karschin et al. (1998) showed that glial cells with glial fibrillary acidic protein (GFAP) expression show a high level of mRNA for Kir6.2, using single-cell PCR techniques. Further, Zhou et al. (2002) showed moderate to intense expression of Kir6.2 protein in glial cells, supported by localization of Kir6.2 mRNA. Indeed, they reported that some glial cells immunopositive for Kir6.2 were simultaneously GFAP immunopositive, suggesting that some astrocytes do