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Research Report

Chronic hypoxia in the human neuroblastoma SH-SY5Y causes reduced expression of the putative α-secretases, ADAM10 and TACE, without altering their mRNA levels

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Abbreviations:
AD, Alzheimer's disease
Aβ, amyloid-β-peptide
ADAM10, a disintegrin and metalloproteinase 10
ADAM17, a disintegrin and metalloproteinase 17
APP, amyloid precursor protein
BACE1, β-amyloid converting enzyme
CH, chronic hypoxia
sAPPα, soluble α-secretase derived N-terminal fragment of APP
TACE, TNF-α-converting enzyme

ABSTRACT

Alzheimer's disease is more frequent following an ischemic or hypoxic episode, with levels of β-amyloid peptides elevated in brains from patients. Similar increases are found after experimental ischemia in animals. It is possible that increased β-amyloid deposition arises from alterations in amyloid precursor protein (APP) metabolism, indeed, we have shown that exposing cells of neuronal origin to chronic hypoxia decreased the secretion of soluble APP (sAPPα) derived by action of α-secretase on APP, coinciding with a decrease in protein levels of ADAM10, a disintegrin metalloprotease which is thought to be the major α-secretase. In the current study, we extended those observations to determine whether the expression of ADAM10 and another putative α-secretase, TACE, as well as the β-secretase, BACE1 were regulated by chronic hypoxia at the level of protein and mRNA. Using Western blotting and RT-PCR, we demonstrate that after 48 h chronic hypoxia, such that sAPPα secretion is decreased by over 50%, protein levels of ADAM10 and TACE and by approximately 60% and 40% respectively with no significant decrease in BACE1 levels. In contrast, no change in the expression of the mRNA for these proteins could be detected. Thus, we conclude that under CH the level of the putative α-secretases, ADAM10 and TACE are regulated by post-translational mechanisms, most probably proteolysis, rather than at the level of transcription.

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1. Introduction

A characteristic feature of Alzheimer's disease (AD) is the accumulation, in neuronal senile plaques aggregates of a 40- to 42-amino-acid peptide, the amyloid β peptide (Aβ), derived from the amyloid precursor protein (APP) by the sequential action of β- and γ-secretases (De Strooper and Annaert, 2000; Ling et al., 2003; Mattson, 1997; Selkoe, 2001). In an alternate pathway, APP is cleaved within the Aβ-domain by α-secretase to form a large (~100 kDa) N-terminal fragment sAPPα which is secreted into the external medium. Thus, processing of APP by the α-secretase pathway precludes the formation of Aβ (De Strooper and Annaert, 2000; Mattson et al., 1993; Selkoe, 2001) and in addition gives rise to sAPPα which has neuroprotective effects (Furukawa et al., 1996; Mattson et al., 1993; Smith-Swintosky et al., 1994). One hypothesis to account for the neuronal damage associated with AD is that processing of APP by the β- and γ-secretase pathway puts neurones at risk due to the decrease in the formation of neuroprotective sAPPα (Mattson et al., 1993). Thus, changes in cellular regulation that lead to a decrease in the α-secretase pathway could accelerate the development of AD either due to an increased production of Aβ or a decrease in sAPPα leading to increased neurotoxicity.

The incidence of AD is notably increased following exposure to periods of cerebral ischemia, for example, in patients with a previous history of cardiovascular disease including arrhythmia and stroke (Kalaria, 2000; Kokmen et al., 1996; Moroney et al., 1996; Tatemichi et al., 1994). APP and Aβ concentrations are increased in postmortem samples of human brain which have been subjected to mild and severe ischemia as well as in animals exposed to ischemia (Abe et al., 1991; Jendroska et al., 1997; Kalaria et al., 1993; Kogure and Kato, 1993; Koistinaho et al., 1996; Tomimoto et al., 1994; Yokota et al., 1996). These data suggest an alteration in the balance of APP processing in favor of the β- and γ-secretase pathway in response to ischemic stress. Thus, ischemic episodes provide an example of cellular stress modifying APP processing. The exact mechanisms underlying this shift in APP processing are unknown. However, recent studies in neuronal cell lines provide clear evidence that a period of chronic hypoxia (CH) can shift processing of APP towards the β/γ-secretase pathway, thus leading to an increase in Aβ formation and a decrease in sAPPα secretion (Green and Peers, 2001; Taylor et al., 1999; Webster et al., 2002, 2004). Additional support is provided by a study (Nalivaeva et al., 2003) which reported that there was a significant decrease in secretion of sAPPα from the sensorimotor cortex of rats subjected to prenatal hypoxia.

We have already shown that exposing the human neuroblastoma SH-SY5Y cell line to 24 h CH leads to a decrease in both constitutive and muscarine-enhanced processing of APP by the α-secretase pathway (Webster et al., 2002, 2004) and have shown that the ERK1/2 pathway is an intracellular mitogen-activated protein kinase (MAPK) signaling pathway crucial to the muscarinic regulation of sAPPα formation in this cell line (Canet-Aviles et al., 2002). In addition, we have shown that the effect of hypoxia on sAPPα is not related to an effect of hypoxia on ERK1/2 activation, nor to the alterations in the stress-activated protein kinases p38MAPK or JNK nor altered expression of APP (Webster et al., 2002, 2004).

On the basis of this work, we consider the most likely mechanism by which CH inhibits sAPPα secretion is through decreasing the activity of α-secretases at the level of protein and possibly through altering transcription. ADAM10, a disintegrin metalloprotease and TNF-α-converting enzyme (TACE, also known as ADAM17), are two strong candidates to be the α-secretase (Allinson et al., 2004; Buxbaum et al., 1998; Lammich et al., 1999). We have shown that ADAM10 protein levels are decreased following 24 h CH in SH-SY5Y cells (Webster et al., 2002, 2004). It is not known at the present time whether TACE might be regulated following chronic hypoxia, although one group has reported that TACE protein levels are increased in rat primary cortical neurones, or brain slices following a shorter period of ischemia induced by oxygen and glucose deprivation (Hurtado et al., 2001, 2002; Romera et al., 2004). It is also possible that if CH increased the levels and activity of BACE, there would be decreased sAPPα due to increased entry of APP into the β/γ-secretase pathway. Wen et al. (2004) have shown that ischemia in rats causes an increase in BACE1 protein levels. However, no data are available on the effect of chronic hypoxia on BACE expression in cell lines.

The aim of this study was to provide more detail of the mechanisms underlying the CH-induced reduction in sAPPα in SH-SY5Y cells. Thus, since regulation of enzyme levels might involve regulation of transcription or mRNA degradation, we determined whether CH affected the mRNA levels of ADAM10, TACE and BACE1. In addition, we extended our previous work on ADAM10 to include the effect of CH on protein levels of the α-secretase candidate TACE and the β-secretase BACE1.

2. Results

2.1. *Western blotting*

Incubation of SH-SY5Y cells under conditions of CH for 48 h decreased muscarine-stimulated sAPPα secretion by between 30 and 40% (data not shown), confirming a loss of α-secretase activity as previously reported for 24 h CH (Webster et al., 2002, 2004).

Under normal conditions SH-SY5Y cells express both the pro- and active forms of ADAM10 and TACE (Fig. 1). Thus, 2 bands of molecular weights between 100 and 85 kDa, corresponding to bands observed in rat brain homogenate, were detected when cell extracts of SH-SY5Y were probed with antibody against ADAM10 (Fig. 1A). After 48 h CH, expression of ADAM10 based on average density of both bands on the Western blots fell to 37.4 ± 9.6% of control levels ($n = 3$). This decrease was statistically significant ($t = 6.627$, $df = 2$, $P = 0.022$ paired t test).

When cell extracts of SH-SY5Y were probed with antibody against TACE, multiple bands were observed. Major bands were observed at ca. 150, 120 and 110 kDa in normoxic cells (Fig. 1B). Treatment of SH-SY5Y cells to CH for 48 h appeared to have little effect on the 150-kDa band but decreased the pro-TACE (120 kDa) band to 9.0 ± 9.0% of control levels compared with a much smaller decrease of the 110-kDa active TACE band (75.2 ± 7.1% of control levels, $n = 3$). The overall decrease in TACE expression was to 59.5 ± 9.0% of controls ($n = 3$) based