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Astrocytic transactivation by α_{2A}-adrenergic and 5-HT_{2B} serotonergic signaling

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ABSTRACT

EGF receptor transactivation has been known for more than ten years. It is a signal pathway in which a G-protein-coupled receptor (GPCR) signal leads to release of a growth factor, which in turn activates the EGF receptor-tyrosine kinase in the same or adjacent cells. Astrocytes express a number of GPCRs and play key roles in brain function. Astrocytic transactivation is of special interest, since its autocrine effect may regulate gene expression and alter cell functions in the cells themselves and its paracrine effect may provide additional opportunities for cross-talk between astrocytes and their neighbors, such as neurons. The signal pathways of EGF transactivation are complicated. This does not only apply to the pathways leading to shedding of growth factor(s), but also to the downstream signal pathways of the EGF receptor, i.e., MAPK and PI3K. The latter may vary according to the type of growth factor released, the sites of tyrosine phosphorylation on the EGF receptor, and the duration of the phosphorylation. Using primary cell cultures we have found that dexmedetomidine, a specific α_2-adrenergic receptor, induced shedding of HB-EGF from astrocytes, which in turn transactivated EGF receptors and stimulated astrocytic c-Fos and FosB expression. At the same time released HB-EGF protected neurons from injury caused by H_2O_2. We have also confirmed dexmedetomidine transactivation in the brain in vivo. EGF transactivation by 5-HT_{2B} receptor stimulation was responsible for up-regulation of $cPLA_2$ in astrocytes by fluoxetine, an antidepressant and inhibitor of the serotonin transporter, which also is a specific 5-HT_{2B} agonist.

1. Introduction

Astrocytes contribute ~20% of cell volume in the brain in vivo (for review, see Hertz, 2008), probably somewhat less in monkey and rat brain, and somewhat more in human brain, where the glia/neuron ratio is higher and the astrocytes are larger and more complex (Pelvig et al., 2008; Oberheim et al., 2009). They express a multitude of receptors for neurotransmitters (Birkenhäger et al., 2004). Among these receptors, α_{2A}-adrenergic receptors (Peng et al., 2003; Li et al., 2008a; Du et al., 2009b), 5-HT_{2B} and 5-HT_{2C} receptors (Li et al., 2007, 2008b, 2010), vasopressin V1 receptors (Du et al., 2008), mGluR5 (Peavy et al., 2001), thrombin receptors (Daub et al., 1997) and P2Y receptors (Daub et al., 1997) have been found to be involved in EGF receptor transactivation in astrocytes (for review, see Peng, 2004). In the transactivation process stimulation of G protein-coupled receptors leads to metalloproteinase-catalyzed shedding of an EGF receptor agonist, which stimulates EGF receptors (EGFRs) on the same cell or its neighbor(s). This stimulation causes phosphorylation of receptor tyrosine kinases (RTKs), which activates two major intracellular signaling pathways, mitogen activated protein (MAP) kinases, including extracellular regulated kinases 1 and 2 ($ERK_{1/2}$), and phosphatidylinositol-3-kinase (PI3K).

During recent years, we have been interested in EGFR transactivation in astrocytes, both signalling pathways and functional significance. There are four topics that have to be addressed (i) the intracellular signal pathways leading to metalloproteinase activation; (ii) the specific metalloproteinase that has been activated, and the specific growth factor(s) shed from cell membranes; (iii) how the EGFR is phosphorylated and (iv) which of the intracellular signal pathways downstream of EGFR is involved in functional changes in the cells, including effects of chronic exposure. Because of the existence of multiple receptors and complexity of signal pathways in the cells, EGFR transactivation has usually been studied in transfected cell lines. Nevertheless, full understanding of the functional significance of the convergence of G-protein-coupled receptors and EGFR under physiological and pathophysiological conditions will eventually rely on studies with intact animals. Compared to cell lines, cells in

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primary cultures are more similar to their counterparts in vivo, regarding the types of receptor expressed, the signal pathways activated and the functional changes by the stimulations. With astrocytes in primary cultures we have demonstrated the requirement for EGFR transactivation in (i) the neuroprotective effect of dexmedetomidine, an agonist of the α_{2A}-adrenergic receptor (Peng et al., 2003; Li et al., 2008a); (ii) regulation of gene expression by stimulation of 5-HT_{2B} receptor (Li et al., 2007, 2008b, 2010); and (iii) cell volume regulation by the V1 receptor (Du et al., 2008), as well as cell volume response to an elevated extracellular potassium ion concentration (Du et al., 2009a). In this paper, we will focus on the first two receptors.

2. EGFR transactivation

2.1. Receptors and ligands

EGFRs are widespread in brain (Prenzel et al., 2001). There are four EGF receptors, HER1, HER2, HER3 and HER4 (ErbB1-ErbB4) (Prenzel et al., 2001). Astrocytes express HER1 and maybe a small amount of HER4 (Pinkas-Kramarski et al., 1997; Kornblum et al., 1999). The stimulation of EGFR causes phosphorylation of RTKs and activation of MAP kinases, including $ERK_{1/2}$, and PI3K. Seven different members of the EGF growth factor superfamily have been identified (EGF, transforming growth factor-α (TGF-α), heparin-binding EGF (HB-EGF), amphiregulin, betacellulin, epiregulin and epigen) (Harris et al., 2003). Only the first four have been demonstrated in brain tissue (Birecree et al., 1991; Nakagawa et al., 1998; Falk and Frisén, 2002; Carrasco et al., 2003; Lu et al., 2005), and EGF, HB-EGF and TGF-α have been identified in mouse astrocytes in primary cultures (Du et al., 2007). Using specific antibody and ELISA method we detected HB-EGF release from astrocytes (Shan et al., 2009). HB-EGF is expressed at high level in brain in vivo at early developmental stages, and declines thereafter (Opanashuk et al., 1999). However, some HB-EGF remains in the cerebellum (Nakagawa et al., 1998), and more recently it has also been demonstrated in other regions, including cerebral cortex (Hagihara et al., 2005). The present observations suggest that it may have important roles in adult brain. TGF-α is found both in neurons and astrocytes of the adult brain (Ojeda et al., 1992), and amphiregulin has also been demonstrated in brain (Falk and Frisén, 2002). EGF expression in postnatal brain is rare (Kawahara et al., 1999), and expression of betacellulin and epiregulin have not been demonstrated in the central nervous system tissue so far.

TGF-α binds to the EGFR (ErbB1) receptor but not to the ErbB4 receptor, whereas HB-EGF binds to both of these receptors (Beerli and Hynes, 1996; Raab and Klagsbrun, 1997; Jones et al., 2003). According to studies in transfected cell lines that express either an individual receptor or a combination of receptors, binding of HB-EGF to those two receptors may indirectly activate other HER (ErbB) family members (Raab and Klagsbrun, 1997).

The EGFR has a number of tyrosine phosphorylation sites. We have been working with Y1173, Y992, Y845, Y1045 and Y1068 using commercially available specific antibodies. Addition of EGF at 10 ng/ml induces phosphorylation at all these five sites in primary cultures of astrocytes (Fig. 1A). Y1173, Y992 and Y1045 are autophosphorylation sites, but Y1173 is the major site and Y992 a minor one. Autophosphorylation is a process in which ligand-dependent receptor dimerization leads to an activation of its cytoplasmic kinase domain and the activated receptor phosphorylates itself at multiple specific tyrosine residues. Autophosphorylation of Y1173 and Y992 are essential for receptor kinase activity, but phosphorylation of Y1045 is related to receptor endocytosis and degradation (Oksvold et al., 2003; Grøvdal et al., 2004). Y845 is a Src phosphorylation site, activated in a bidirectional interaction between EGFR and Src (Bromann et al., 2004). Its phosphorylation can be inhibited by PP1, an inhibitor of Src, but not by AG1478, an inhibitor of receptor tyrosine kinase (Reinehr et al., 2005). Y845 is also associated with receptor down-regulation and endocytosis, but it is not related to receptor activity (Kasai et al., 2005; Han et al., 2006; Frey et al., 2006; Pennock and Wang, 2008; Demory et al., 2009). Y1068 is not phosphorylated in the brain in vivo (Du et al., 2009b) and in normal astrocytes unless stimulated by EGF (Fig. 1). The only difference between phosphorylation sites in cultured astrocytes (Fig. 1) and in the brain in vivo (Du et al., 2009b) seems to be that the level of phosphorylation at Y1173 in control samples is higher in the brain in vivo than in astrocytes. It might therefore also be caused by other cell components in the brain in vivo. Only small differences were found in phosphorylation profile of the EGFR by HB-EGF and TGF-α factors in a human carcinoma cell line (Guo et al., 2003).

2.2. Transactivation

In addition to activation by growth factors of the EGF family, the EGF receptor can be transactivated via $G_{i/o}$ or G_q protein-coupled receptors (Pierce et al., 2001; Peng, 2004; Li et al., 2008b). Transactivation is a pathway in two-stages as has been elegantly shown in transfected COS-7 cells (Pierce et al., 2001). In the first stage the βγ subunits of the activated, heterotrimeric G_i protein lead, via activation of cytosolic Src tyrosine kinases, to proteolytic, metalloproteinase-mediated 'shedding' of heparin-binding epithelial growth factor (HB-EGF) from its extracellular transmembrane-spanning HB-EGF precursor; in the second stage the shedded HB-EGF 'transactivates' EGFRs in the same and adjacent cells in conventional manners, i.e., the RTK of the EGFR is phosphorylated and internalized, contributing directly to Ras- and Raf-dependent ERK phosphorylation, which in these cells also was dependent upon Src. The signal pathways leading to activation of metalloproteinase(s) may vary in different cell types and after stimulation of different receptors.

Metalloproteinases (MPs) form a large family consisting of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase group (ADAMs). Among 25 members of the MMP family and 34 members of the ADAM family, 15 (ADAM10, ADAM17, ADAM22, ADAM23, MMP2, MMP3, MMP9, MMP11, MMP13, MMP14, MMP15, MMP16, MMP17, MMP24, MMP25) are expressed in the brain in vivo and also in astrocytes (Hu et al., 2009). Compared with brain tissue, astrocytes have less MMP3, MMP15, MMP24 and MMP25 (Hu et al., 2009). Extensive work needs to be done in order to identify the specific metalloproteinases involved in EGFR transactivation in different types of cells and by different stimulatory factors on account of the large family of the enzyme and lack of specific inhibitors. GM6001 that is widely used experimentally is a broad-spectrum, Zn-binding metalloproteinase inhibitor (Grobelny et al., 1992). Experiments with transfected cells and knockout mice have suggested that the membrane-bound precursors of EGF and betacellulin are substrates of ADAM10, but precursors of TGF-α, epiregulin, amphiregulin and HB-EGF substrates of ADAM17 (Sahin et al., 2004). In contrast, a study by Horiuchi et al. (2007) showed that phorbol ester activated ADAM10, but Ca^{2+} influx ADAM17, and that both of them stimulated "shedding" of TGF-α and amphiregulin. In the family of metalloproteinases, ADAM17 (tumor necrosis factor-α-converting enzyme (TACE)) has been extensively studied on account of the potential clinical application of its inhibitors in tumors and inflammatory and vascular diseases (for review, see Arribas and Esselens, 2009). The involvement of ADAM17 has been demonstrated in P2Y receptor-mediated TGF-α shedding from fibroblasts and CHO cells (Myers et al., 2009), in ATP-dependent HB-EGF shedding from human corneal cells (Yin and Yu, 2009), in angiotensin II-induced EGFR transactivation in hepatocellular carcinoma cells (Itabashi et al., 2008), in shedding of HB-EGF from