PERSPECTIVE

G protein-coupled estrogen receptor 1 (GPER) activation triggers different signaling pathways on neurons and astrocytes

Estradiol (E2) is the most potent and prevalent form of estrogen, a well-known hormone that regulates multiple tissues and functions in humans. In the brain, E2 regulates processes as diverse as learning, memory, cognition, mood, as well as neurodevelopment and neurodegeneration. The actions of E2 are mediated by classical estrogen receptors (ERs; α and β), and by the G protein-coupled estrogen receptor 1 (GPER or GPR30) (Prossnitz and Arterburn, 2015). Classical ER are predominantly present in the nucleus and cytoplasm, with less than 2% present on the plasma membrane, and mediate genomic cellular effects that occur in the time frame of hours to days (Prossnitz and Arterburn, 2015). GPER is expressed on the plasma membrane, and on intracellular membranes of the endoplasmic reticulum and Golgi apparatus, and mediates rapid estrogen-induced effects that occur in the time frame of seconds to minutes (Prossnitz and Arterburn, 2015).

The cellular and molecular effects triggered by the activation of classical ER on brain cells are well known. However, they cannot explain all the effects induced by E2. It is well established that when E2 binds to classical ER there are modifications in their structure that lead to the formation of homodimers and/or heterodimers (Prossnitz and Arterburn, 2015). These dimers bind to estrogen response elements (EREs) in the DNA and recruit other components of the transcriptional machinery, leading to gene expression (Prossnitz and Arterburn, 2015). Nevertheless, there are also cytoplasmic signaling events or transduction cascades initiated on the plasma membrane, classified as non-genomic, such as, modulation of intracellular calcium, the production of cyclic adenosine monophosphate (cAMP), regulation of phosphoinositide 3-kinase (PI3Ks), and mitogen-activated protein kinases (MAPKs)/extracellular signal-regulated kinases (ERKs), being these non-genomic pathways associated with the activation of GPER (Prossnitz and Arterburn, 2015). Additionally, the rapid signaling events initiated by GPER upregulate the expression of genes such as c-fos, cyclin D2 and Bcl-2 (Prossnitz and Arterburn, 2015).

The signaling mechanisms activated by GPER in neural tissues are not completely understood, and the available data focus mainly on the effects triggered in neurons (Evans et al., 2016). GPER activation in neurons promotes the activity of pro-survival kinases such as PI3K/Akt (Tang et al., 2014; Cheng et al., 2016) and ERK (Tang et al., 2014), and attenuates the pro-apoptotic pathway JNK (Tang et al., 2014; Cheng et al., 2016). Besides that, the GPER agonist G1 also induces the activation of adenylyl cyclase and the consequent rise in cAMP levels in a dose-dependent manner in neurons (Evans et al., 2016). This intracellular messenger has a preponderant role in several signal transduction cascades and thus it is likely that, in addition to the above mentioned signaling pathways, others may also be influenced by the selective activation of GPER.

In contrast, we have previously demonstrated that the selective activation of GPER in astrocytes with G1 induces their apoptosis, while having no impact on neuronal survival (Roque et al., 2018). The differential effects are due to the activation of different signaling pathways on each type of cell (Roque et al., 2018). In astrocytes, but not in neurons, G1 induces the activation of phospholipase C (PLC) and a rise in intracellular calcium levels that triggers astrocytes apoptosis (Roque et al., 2018). These pro-apoptotic effects of G1 were also observed in other cell types, such as the vascular smooth muscle cells (Ding et al., 2009). In this case, the effects were associated with the activation of ERK and inhibition of PKA (Ding et al., 2009). Moreover, GPER could have the ability to control microglial reactivity through the decrease of phagocytic activity, inducible nitric oxide synthase expression and nitric oxide (NO) release (Mendes-Oliveira et al., 2017).

The existence of cell type-specific signaling pathways in response to activation of estrogen receptors was already proposed years ago. Mhyre and Dorsa (2006) reported that the activation of classical ER triggers different rapid signaling pathways in neurons and astrocytes. In neuronal cells the activation of classical ER is coupled to the activation of the MAPK and cAMP response element-binding protein (CREB) pathways (Mhyre and Dorsa, 2006). Conversely, in astrocytes E2 does not increase the phosphorylation of MAPK or CREB pathways, but instead activates signaling pathways leading to inhibition of cAMP response elements (CRE) and CRE-mediated transcription (Mhyre and Dorsa, 2006). Since classical ER and GPER activate common signaling pathways (Prossnitz and Arterburn, 2015), we hypothesize that the same cell type-specific response may be induced by GPER selective activation. Besides the PLC pathway (Roque et al., 2018), other signaling pathways, involving cAMP and/or MAPK, may be involved in these cell type-specific signaling mechanisms.

It is known that MAPK activation provides cell type-specific signals important for cellular differentiation, proliferation, and survival, and that cAMP has divergent effects on MAPK activity (Dugan et al., 1999; Qiu et al., 2000). Activation of the MAPK pathway by cAMP requires the presence of B-raf, and neurons, but not glial cells, express it (Dugan et al., 1999; Qiu et al., 2000). Thus, it is possible that this difference will lead to specific actions of cAMP on the MAPK pathway in these two cell populations. Indeed, this is supported by studies showing that cAMP activates the MAPK pathway in neurons (Dugan et al., 1999; Qiu et al., 2000). In astrocytes, cAMP reduces MAPK activity (Dugan et al., 1999; Qiu et al., 2000), but transfection of B-raf enabled MAPK activation in response to cAMP in these cells (Dugan et al., 1999; Qiu et al., 2000). These effects on MAPK pathway were also associated to the reduction of cell growth (Dugan et al., 1999). Although these observations suggest that neurons and astrocytes respond differently to GPER selective activation due to a differential activation of the MAPK pathway, it is necessary to address directly this hypothesis by evaluating the modulation of MAPK pathway upon selective activation of GPER.

As previously mentioned, the activation of the PI3K/Akt pathway by GPER agonists was consistently associated with protective effects mediated by this receptor (Prossnitz and Arterburn, 2015), with several studies showing that GPER promotes neuronal survival through activation of this pathway (Tang et al., 2014; Cheng et al., 2016). Regarding astrocytes, there is no data about the activation of the PI3K/Akt pathway by GPER. However, it was demonstrated that cAMP inhibits the PI3K/Akt pathway in astrocytes (Wang et al., 2001; Sugimoto et al., 2011), being these effects reversed by the constitutively active form of PI3K (Wang et al., 2001). This inhibition was associated with the presence of several apoptotic markers, such as morphological changes, increase of cleaved caspase-3, condensation and fragmentation of nuclei, and a decrease in the number of cells (Sugimoto et al., 2011). These data suggest a differential effect of cAMP on neurons and astrocytes caused by a different regulation of the PI3K/Akt pathway on these two cell populations. Again, this needs to be evaluated experimentally.

In summary, existing data indicates that selective activation of GPER triggers different signaling pathways in neural cells resulting in cell type-specific responses (**Figure 1**). Activation of GPER in

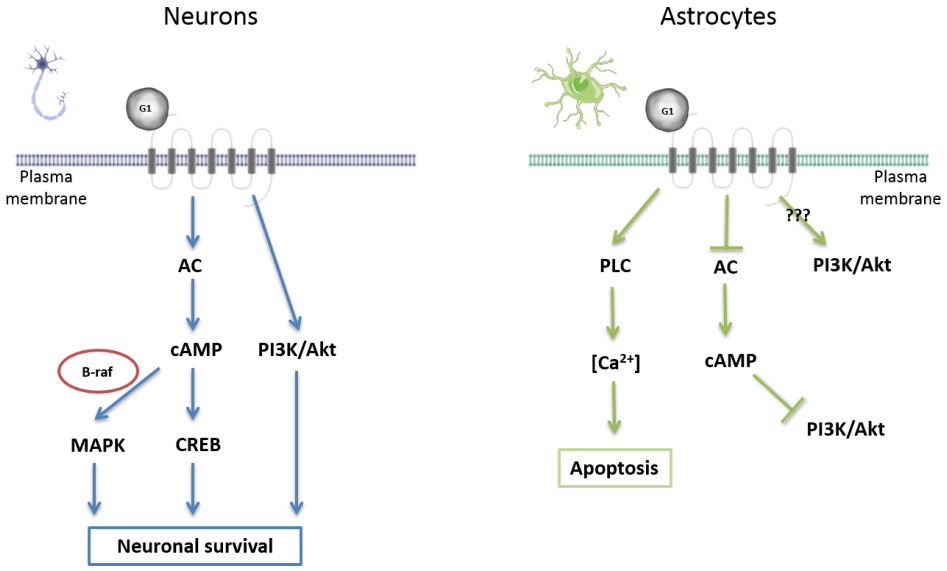

Figure 1 Cell type-specific signaling pathways activated by GPER on neurons and astrocytes.
On neuronal cells the activation of GPER is associated to an increase of neuronal survival, *via* the increase of cAMP levels and the activation of MAPK and CREB pathways, and *via* the activation of PI3K pathway. On astrocytes the activation of GPER is associated to a reduction of cAMP levels and to cell death due to the activation of PLC pathway and consequent rise in intracellular calcium levels. GPER: G protein-coupled estrogen receptor 1; AC: adenylate cyclase; CREB: cAMP response element-binding protein; cAMP: cyclic adenosine monophosphate; GPER: G protein-coupled estrogen receptor 1; [Ca^{2+}]: intracellular calcium; MAPK: mitogen-activated protein kinase; PI3K: phosphoinositide 3-kinase; PLC: phospholipase C; Akt: protein kinase B.

neurons is associated with the activation of pathways that lead to the promotion of cell survival. In astrocytes, the signaling pathways activated by which GPER exerts its beneficial role in neurons are either absent, or inhibited. Considering the crucial role of glial cells in neuronal physiology, it is likely that any condition that interferes with the normal astrocytic and microglial function affects neuronal physiology. Since the number of studies on the actions of GPER in glial cells is still very limited, it is of utmost importance to deepen the analysis of the effects triggered by GPER activation on this cell population. Clarification of these cell type-specific signaling mechanisms will help to elucidate the potential, and possibly selective, protective role of GPER activation in brain pathologies and neurodegenerative disorders.

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