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Research Report

GPR30 activation is neither necessary nor sufficient for acute neuroprotection by 17β-estradiol after an ischemic injury in organotypic hippocampal slice cultures

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ABSTRACT

In this study, we investigated the role of GPR30 in 17β-estradiol- (E2) mediated neuroprotection after an ischemic injury in an organotypic hippocampal slice culture (OHSC) model. We report that after oxygen–glucose deprivation (OGD), a physiological concentration of 100 pM E2 provided the greatest significant reduction in cell death while supra-physiological levels were less effective. The canonical estrogen receptor (ER) inhibitor ICI 182,780 completely abrogated the therapeutic effect of E2 while the GPR30 antagonist G−15 effected a slight but not significant reduction in neuroprotection. Only supra-physiological levels of E2 led to significantly increased phosphorylation of Akt and Erk which are well known downstream effects of GPR30 activation. We conclude that GPR30 activation may facilitate acute E2 mediated neuroprotection after OGD, but is neither necessary nor sufficient.

1. Introduction

In the United States, stroke is the fourth leading cause of death resulting in more than 130,000 deaths per year and is also the leading cause of serious long-term disability (Heron, 2011; Roger et al., 2012). Current methods of treating acute stroke are focused on restoration of blood flow to the brain (Saver, 2006). During stroke, the ischemic lesion propagates from its core that was previously perfused by the occluded vessel. The surrounding penumbral area is deprived of blood, causing cell death, until the clot is removed (Astrup et al., 1981). Understanding the mechanism of delayed cell death in the penumbral area after an ischemic stroke may lead to pharmacological interventions to ameliorate further, unnecessary brain damage and consequent disability. Recent studies have indicated that E2 is neuroprotective when administered after an ischemic injury, but the mechanism by which E2 exerts neuroprotection is not fully understood.

For over two decades, the most potent and naturally occurring form of estrogen, 17β-estradiol, has been investigated

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as a neuroprotective therapy for stroke (Paganini-Hill et al., 1988; Paganini-Hill, 1995; Simpkins and Singh, 2008). In early animal studies, E2 was neuroprotective when administered before cerebral ischemia in vivo, which has led many to investigate its mechanism of prophylaxis (Simpkins et al., 1997). The neuroprotective effects of E2 have been attributed to anti-oxidant effects (Behl et al., 1995; Numakawa et al., 2007), changes in NMDA channels (Liu and Zhao, 2013), changes in vasculature (Xia et al., 2007), and anti-apoptotic effects (Simpson et al., 2005). However, far fewer studies have investigated estrogen's role as a post-ischemic therapy, and no consensus has been reached on whether E2 is beneficial when administered acutely after ischemia (Yang et al., 2000).

GPR30, an orphan G-protein coupled receptor, is a recently discovered and controversial target of E2 which has near immediate physiological response in neurons (Carmeci et al., 1997; Liu et al., 2012; Revankar et al., 2005). The rapid response of GPR30 to E2 has led to the speculation that GPR30 may be responsible for the acute benefits of E2 when administered after an ischemic injury. While several groups have shown through immunofluorescence binding experiments (Funakoshi et al., 2006) and activation of downstream pathways (Filardo et al., 2002; Prossnitz and Barton, 2009; Revankar et al., 2005) that E2 does bind to and activate GPR30, the concentrations needed for these effects (>1 nM) are far from physiological levels (10–120 pM) (Strom et al., 2010, 2011). Other groups have shown no binding affinity of E2 for GPR30 which suggests these effects are off-target (Otto et al., 2008). Also, the interplay, if any, between classical ERs and GPR30 is not well understood (Lebesgue et al., 2009). However, because it is a G-protein coupled receptor and can elicit physiological responses within seconds (Revankar et al., 2005), GPR30 is an attractive target as the potential mediator of acute E2 neuroprotection following ischemia.

In this study, we used an in vitro model of ischemia to determine the role of GPR30, if any, in acute E2 mediated neuroprotection. OGD is a well-established and reproducible in vitro model of ischemia (Sundstrom et al., 2005), which eliminates many confounds of in vivo models including the influence of the route of drug administration, changes in blood flow, partitioning effects of the blood brain barrier, and injury reproducibility. We tested the efficacy of both physiological and supra-physiological doses of E2 as a neuroprotective agent after OGD. Utilizing agonists and inhibitors to classical ERs and

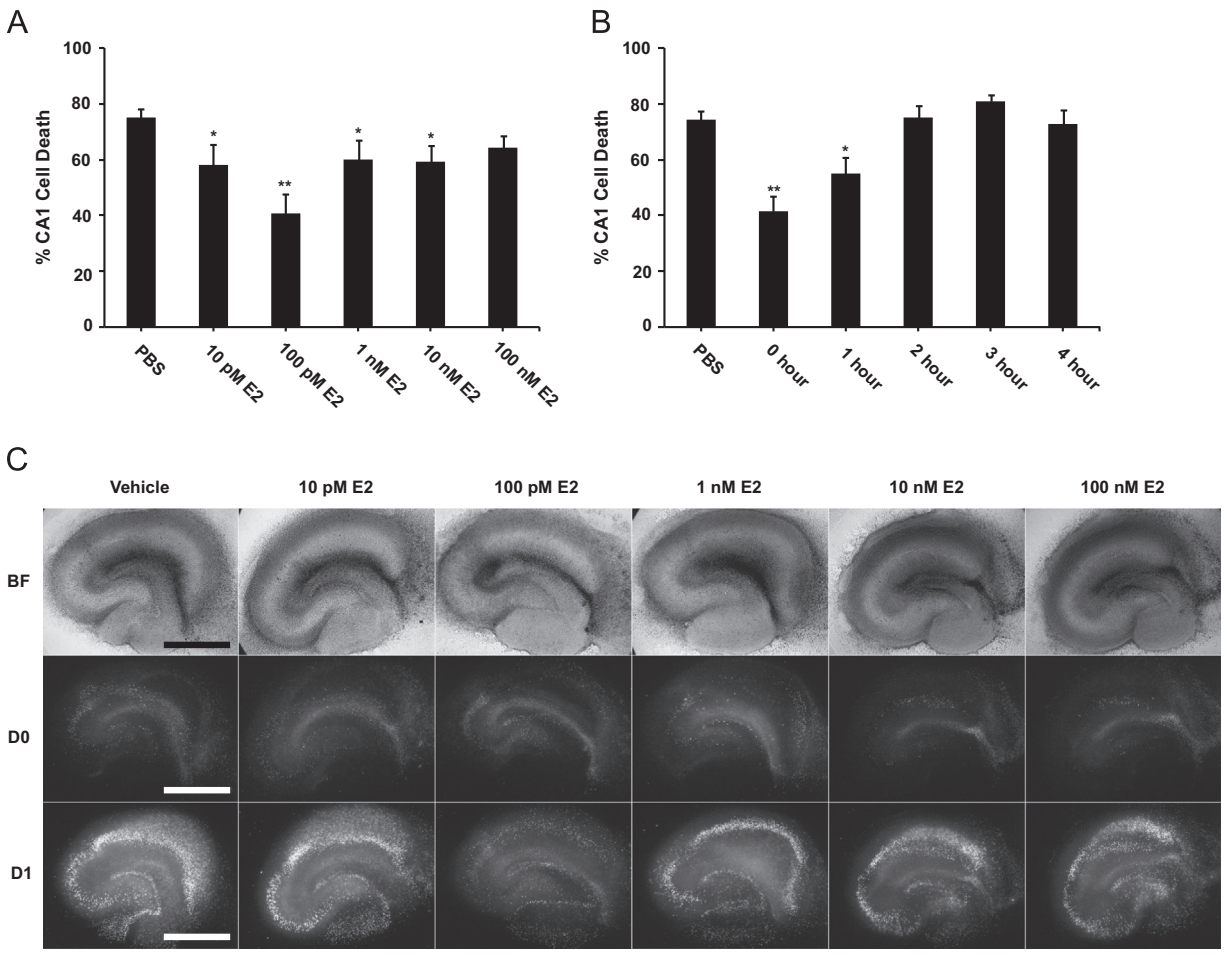

Fig. 1 – E2 is neuroprotective in rat OHSCs exposed to OGD. (A) PBS or E2 was administered immediately after OGD injury, and cell death was quantified 24 h later in the CA1 region of the hippocampus using propidium iodide (PI). $^{*}P<0.05$, $^{**}P<0.01$ versus vehicle injured control. (B) Cell death was quantified 24 h after OGD injury with vehicle or 100 pM E2 added at indicated time points after injury. $^{*}P<0.05$, $^{**}P<0.01$ versus vehicle injured control. (C) Representative bright-field and PI images before injury and PI images 24 h after injury for dose response of E2. Scale bars=1 mm.