SYNOPSIS

Title of thesis: G-Protein coupled Estrogen Receptor (hGPER) mediates action of 17β-estradiol on hTREK-1 potassium channel

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TREK-1 is a two-pore domain potassium channel that contributes to maintenance of the resting membrane potential of a cell. TREK-1 is involved in several physiological and pathophysiological conditions like nociception, anaesthesia, epilepsy, ischemia and depression. Activity of TREK-1 is modulated by a number of physical and chemical stimuli including the activation of G-protein coupled receptors by several neurotransmitters and hormones. An important modulator of neuronal activity and function is 17β-estradiol, which by acting through its classical receptors ERα and ERβ, can bring about genomic changes in the cell. 17β-Estradiol can also act through membrane receptors like the G-protein coupled estrogen receptor (GPER) and activate intracellular signaling pathways. Several neuroprotective effects of 17β-estradiol in epilepsy, ischemia and diseases like Alzheimer's and Parkinson's is mediated through activation of GPER. 17β-Estradiol is also known to modulate the activity of several ion channels in a non-genomic manner, thus, regulating the neuronal function. Of the different membrane ionic channels, the leak potassium channel TREK-1 is implicated in neuroprotection since their activation hyperpolarizes the membrane of neurons and astrocytes and reduces neuronal excitability. However, it is not known whether 17β-estradiol can physiologically modulate the activity of TREK-1 channels and use this as an additional mechanism to mediate neuroprotection.

In the present study, using single-channel cell-attached patch-clamp electrophysiology in HEK293 cells, we show that 17β-estradiol increases the activity of hTREK-1 by an hGPER-dependent mechanism. The probability of opening of the hTREK-1 channel increased rapidly and irreversibly on application of 17β-estradiol, not directly but only in the presence of hGPER. The potentiation of hTREK-1 activity by 17β-estradiol was mimicked by hGPER

agonist and inhibited by hGPER antagonist, supporting the hGPER-dependence of 17β-estradiol action.

Pharmacological studies demonstrated that the hGPER-mediated potentiation of hTREK-1 by 17β-estradiol occurred in a pertussis toxin-sensitive manner, mediated by the Gβγ subunits. Raising the intracellular cAMP levels reversed the potentiation of hTREK-1 induced by 17β-estradiol suggesting the inhibition of cAMP production in the hGPER-mediated increase of hTREK-1 activity. The hGPER-dependent rise in hTREK-1 activity induced by 17β-estradiol was occluded by inhibition of PKA which indicated that 17β-estradiol action involves inhibition of PKA.

The serines at position 315 and 348 in the C-terminal domain of hTREK-1 are involved in phosphorylation-mediated inhibition of channel activity as known from earlier studies. Mutational studies with S315 and S348 suggested that S348 was the target site for dephosphorylation and potentiation of hTREK-1 by hGPER-mediated action of 17β-estradiol. 17β-Estradiol-induced potentiation of hTREK-1 was abolished on inhibition of serine/threonine phosphatases suggesting the requirement of serine/threonine phosphatases for the action of 17β-estradiol. Thus, the inhibition of PKA acts jointly with the activation of serine/threonine phosphatases to dephosphorylate S348 in the C-terminal domain of hTREK-1 leading to an increase in its activity.

It was known from previous literature that TREK-1 and 17β-estradiol play important roles in neuroprotection. However, the effect of 17β-estradiol on the TREK-1 channel was not explored. The study undertaken as part of this thesis provides the link between 17β-estradiol and TREK-1 activity, giving an insight into a plausible mechanism underlying the several neuroprotective roles of 17β-estradiol.