HEME OXYGENASE ACTIVITY IN IMMORTALIZED HYPOTHALAMIC NEURONS GT1-7

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Heme oxygenase (HO) is a widely distributed antioxidant and anti-inflammatory enzyme responsible for heme degradation into ferrous iron, carbon monoxide (CO), and biliverdin. CO has been identified to play an important role in synaptic plasticity and neuropeptide release. Biliverdin is then reduced to bilirubin, this latter acting as antioxidant and anti-nitrosative moiety. Functional interplay among HO and other pro-inflammatory enzymatic activities, like cyclooxygenase, has been suggested to be relevant among others for tissue damage and aging processes. GT1-7 cells are immortalized hypothalamic neurons characterized by spontaneous electrical activity and pulsatile gonadotropin-releasing hormone (GnRH) release. Various neuropeptides and neurotransmitters modulate these properties in both directions stimulatory and inhibitory, playing a key role in hypothalamic-hypophyseal-gonadal function. The purposes of this study were to investigate, characterize the presence of HO in GT 1-7 cells and describe any effect due to the products of HO enzymatic activity on GnRH release and cell viability. The presence of HO isoforms was determined by Western blotting. HO immunoreactivity was detected at the expected size and increased after prolonged cellular exposure to hemin 10^{-6} M reaching the maximum after 24 hours. Bilirubin production was measured in the incubation medium to confirm enzymatic activity. To investigate the modulatory effects of HO products on GnRH release, GT1-7 cells were studied in static cultures and in a perifusion system, allowing cells to form a tridimensional network on cytodex beads. In both experimental conditions exposure in pulses, from 15 minutes to hours, to the HO substrate, hemin, ranging from 10^{-8} to 10^{-6} M caused a slight increase in GnRH secretion, without changes in peak amplitude and frequency. No differences in LDH activities in the perifusion or incubation media were observed in any condition tested, suggesting a satisfactory maintenance of cell viability and a role of CO in modulating GnRH release. In both perifusion and static cultures studies Sn-PP-IX was able to modulate the slight increase in GnRH secretion. In conclusion HO is expressed and functional in GT1-7 cells providing relevant neuroprotection against oxidative stimuli. GT1-7 cells are a valuable model to further investigate the mechanisms of oxidative and inflammatory damage in neurons.
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