AGE-RELATED DECREASES IN NAD(P)H AND GLUTATHIONE CAUSE REDOX DECLINES DURING GLUTAMATE TREATMENT OF HIPPOCAMPAL NEURONS

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NADH generated by glycolysis and the Krebs cycle is necessary for reduction reactions and to power production of ATP. Both NADPH and reduced glutathione (GSH) are important in protection of cells from oxidative damage. Because neurons from old rats are more susceptible to glutamate toxicity than younger neurons, we sought evidence in cultured hippocampal neurons for 1) age-related changes in NAD(P)H and redox state and 2) susceptibility of NAD(P)H and redox state to glutamate with age. Hippocampal neurons were isolated from rat brains at embryonic day 18, middle-age (9 months) or old-age (22–24 months) and cultured for 8–15 days under common serum-free conditions. Fluorescence for NAD(P)H and FAD was collected simultaneously from single cells. At rest, we observed an age-dependent decline in NAD(P)H, redox ratio (NAD(P)H / FAD) and GSH content. Glutamate addition resulted in an initial short-lived increase in NAD(P)H and redox ratio followed by a larger decline in all ages, but the old-age neurons showed the greatest percentage of decline. The mitochondrial origin of the signal was confirmed by inhibition of NAD(P)H:ubiquinone oxidoreductase by rotenone which increased NAD(P)H fluorescence. Addition of glutamate with rotenone caused an initial increase in NAD(P)H and redox ratio followed by smaller decays, suggesting initial stimulation by the calcium influx of NADH production from mitochondrial dehydrogenases. The present results show an age-related decline in NAD(P)H and redox ratio exacerbated by glutamate. NAD(P)H and GSH deficiencies that persist in our culture model could be key factors in age-related and glutamate induced susceptibility of old–age neurons to excitotoxicity.