The Long-Term Impairment in Redox Homeostasis Observed in the Hippocampus of Rats Subjected to Global Perinatal Asphyxia (PA) Implies Changes in Glutathione-Dependent Antioxidant Enzymes and TIGAR-Dependent Shift Towards the Pentose Phosphate Pathways: Ef

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We have recently reported that global perinatal asphyxia (PA) induces a regionally sustained increase in oxidized glutathione (GSSG) levels and GSSG/GSH ratio, a decrease in tissue-reducing capacity, a decrease in catalase activity, and an increase in apoptotic caspase-3-dependent cell death in rat neonatal brain up to 14 postnatal days, indicating a long-term impairment in redox homeostasis. In the present study, we evaluated whether the increase in GSSG/GSH ratio observed in hippocampus involves changes in glutathione reductase (GR) and glutathione peroxidase (GPx) activity, the enzymes reducing glutathione disulfide (GSSG) and hydroperoxides, respectively, as well as catalase, the enzyme protecting against peroxidation. The study also evaluated whether there is a shift in the metabolism towards the penthose phosphate pathway (PPP), by measuring TIGAR, the TP53-inducible glycolysis and apoptosis regulator, associated with delayed cell death, further monitoring calpain activity, involved in bax-dependent cell death, and XRCC1, a scaffolding protein interacting with genome sentinel proteins. Global PA was induced by immersing fetus-containing uterine horns removed by a cesarean section from on term rat dams into a water bath at 37 °C for 21 min. Asphyxia-exposed and sibling cesarean-delivered fetuses were manually resuscitated and nurtured by surrogate dams. Animals were euthanized at postnatal (P) days 1 or 14, dissecting samples from hippocampus to be assayed for glutathione, GR, GPx (all by spectrophotometry), catalase (Western blots and ELISA), TIGAR (Western blots), calpain

(fluorescence), and XRCC1 (Western blots). One hour after delivery, asphyxia-exposed and control neonates were injected with either 100 ?l saline or 0.8 mmol/kg nicotinamide, i.p., shown to protect from the short- and long-term consequences of PA. It was found that global PA produced (i) a sustained increase of GSSG levels and GSSG/GSH ratio at P1 and P14; (ii) a decrease of GR, GPx, and catalase activity at P1 and P14; (iii) a decrease at P1, followed by an increase at P14 of TIGAR levels; (iv) an increase of calpain activity at P14; and (v) an increase of XRCC1 levels, but only at P1. (vi) Nicotinamide prevented the effect of PA on GSSG levels and GSSG/GSH ratio, and on GR, GPx, and catalase activity, also on increased TIGAR levels and calpain activity observed at P14. The present study demonstrates that the long-term impaired redox homeostasis observed in the hippocampus of rats subjected to global PA implies changes in GR, GPx, and catalase, and a shift towards PPP, as indicated by an increase of TIGAR levels at P14.