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ALTERATIONS IN NEURONAL CONTROL OF BODY WEIGHT AND ANXIETY BEHAVIOR BY GLUTATHIONE PEROXIDASE 4 DEFICIENCY

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Abstract—Elevated levels of oxidative stress and neuronal inflammation in the hypothalamus or ventral midbrain, respectively, represent common denominators for obesity and Parkinson's Disease (PD). However, little is known about defense mechanisms that protect neurons in these regions from oxidative damage. Here, we aimed to assess whether murine Gpx4, a crucial antioxidant enzyme that protects neurons from membrane damage and ferroptosis, is critical for the protection from neuronal inflammation in two distinct pathophysiologic diseases, namely metabolic dysfunction in diet-induced obesity or PD. Gpx4 was deleted from either AgRP or POMC neurons in the hypothalamus, essential for metabolic homeostasis, or from dopaminergic neurons in the ventral midbrain, governing behaviors such as anxiety or voluntary movement. To induce a pro-inflammatory environment, AgRP and POMC neuron-specific Gpx4 knockout mice were subjected to high-fat high-sucrose (HFHS) diet. To exacerbate oxidative stress in dopaminergic neurons of the ventral midbrain, we systemically co-deleted the PD-related gene DJ-1. Gpx4 was dispensable for the maintenance of cellular health and function of POMC neurons, even in mice exposed to obesogenic conditions. In contrast, HFHS-fed mice with Gpx4 deletion from AgRP neurons displayed increased body adiposity. Gpx4 expression and activity were diminished in the hypothalamus of HFHS-fed mice compared to standard diet-fed controls. Gpx4 deletion from dopaminergic neurons induced anxiety behavior, and diminished spontaneous locomotor activity when DJ-1 was co-deleted. Overall, these data suggest a physiological role for Gpx4 in balancing metabolic control signals and inflammation in AgRP but not POMC neurons. Moreover, Gpx4 appears to constitute an important rheostat against neuronal dysfunction and PD-like symptoms in dopaminergic circuitry within the ventral midbrain.

Key words: obesity, Parkinson's disease, DJ-1, hypothalamus, antioxidant, lipid peroxidation.

INTRODUCTION

Metabolic diseases such as obesity and neurodegenerative diseases such as Parkinson's Disease (PD) share a prominent common feature: increased oxidative stress, manifested by a surge in reactive oxygen species (ROS) and inflammatory signaling in neurons within the hypothalamus or ventral midbrain, respectively. Obesity has been associated with increased circulating levels of inflammatory cytokines, which activate ROS-releasing pathogen defense enzymes such as superoxide ($O_2^{\cdot-}$)-producing NADP oxidase in multiple tissues (Erdos et al., 2009; Jaillard et al., 2009). Obesity was further linked with the excessive release of ROS from mitochondria due to nutritional substrate overloading, which results in electrons escaping from the electron transport chain to form $O_2^{\cdot-}$ (Brownlee, 2005). For PD, the role of inflammatory processes in its pathology is firmly established (Moehle and West, 2015; Joers et al., 2016). Mitochondrial dysfunction, high constitutive firing activity and dopamine metabolism itself, which all translate into a constant and high level of ROS, are thought to render dopaminergic neurons of

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Abbreviations: AgRP, agouti related protein; Gpx4, glutathione peroxidase 4; GSH, glutathione; PD, Parkinson's disease; POMC, proopiomelanocortin; SN, substantia nigra.

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the ventral midbrain particularly vulnerable to degeneration (Surmeier et al., 2012; Blesa et al., 2015).

ROS, such as $O_2^{\cdot-}$ or hydrogen peroxide (H_2O_2), induce cellular damage at their site of production, for instance mitochondrial DNA damage or protein oxidation which may trigger the unfolded protein response (Santos et al., 2009; Shokolenko et al., 2009). Moreover, H_2O_2 can form the highly reactive hydroxyl radicals ($OH^{\cdot}$) that attack polyunsaturated fatty acids thus forming alkoxyl/peroxyl radicals and phospholipid hydroperoxides. Lipid peroxidation was associated with severe pathophysiological consequences in multiple peripheral tissues and brain areas, including perturbed membrane structure and function, activated cellular stress signaling and ferroptosis, a non-apoptotic form of cell death (Keaney, 2003; Furukawa et al., 2004; Benani et al., 2007; Yoo et al., 2010; Dixon et al., 2012). There is no enzymatic defense mechanism against highly reactive hydroxyl radicals; solely nutrient-derived antioxidants such as vitamin E provide a certain protection (Pfluger et al., 2004). However, once lipid peroxides are formed, they can be efficiently detoxified by glutathione peroxidase 4 (Gpx4) at the expense of the highly abundant antioxidant glutathione (GSH; Fig. 1a).

Gpx4 is a selenocysteine-containing member of the glutathione peroxidase family with three isoforms transcribed from the same gene: a ubiquitously expressed cytosolic form (cGpx4), and the mitochondrial (mGpx4) and sperm nuclei (snGpx4) forms whose expression is largely restricted to testes. Global germline deletion of mGpx4 and snGpx4 yielded fully viable but infertile mice that displayed perturbed sperm maturation (Conrad et al., 2005; Schneider et al., 2009). In contrast, global Gpx4 deletion evoked early embryonic lethality at day 7.5 (Yant et al., 2003). Mice with germline CAMKIIalpha-driven Gpx4 ablation from the forebrain, while born inconspicuously, developed an atactic gate and fatal hyperexcitable phenotype by postpartum day 13 (Seiler et al., 2008). The essential role of Gpx4 was also corroborated in inducible CAMKIIalpha-driven Gpx4 knockout (KO) mice, which displayed spinal motor neuron degeneration, paralysis and death within 8 days of tamoxifen injection due to exacerbated lipid peroxidation, mitochondrial dysfunction and ferroptosis (Chen et al., 2015).

Mice with Gpx4 haploinsufficiency ($Gpx4^{+/-}$) were viable and fertile when fed standard low-fat diet (Katunga et al., 2015). However, when chronically exposed to high-fat high-sucrose diet (HFHS), $Gpx4^{+/-}$ mice displayed classical symptoms of the metabolic syndrome as well as cardiac hypertrophy and cardiac fibrosis. These metabolic perturbations were associated with increased levels of lipid peroxidation, mitochondrial dysfunction, increased ROS release and the upregulation of pro-inflammatory genes in livers and hearts of obese $Gpx4^{+/-}$ but not wild-type (WT) mice (Katunga et al., 2015). Putative detrimental effects of Gpx4 haploinsufficiency on the CNS were not assessed by Katunga et al. (Katunga et al., 2015). However, earlier reports showed increased lipid peroxidation in the CNS of $Gpx4^{+/-}$ mice and early signs of Alzheimer's Disease such as increased amyloidogenesis (Chen et al., 2008).

Here we aimed to assess the role of Gpx4 in hypothalamic proopiomelanocortin (POMC) and agouti-related protein (AgRP) neurons residing in the arcuate nucleus. These two neuronal subpopulations, which are characterized by neuropeptide production of either POMC or AGRP, sense peripheral nutrients and hormones and govern adaptive metabolic responses to environmental changes (Belgardt et al., 2009; Varela and Horvath, 2012; Denis et al., 2014). Our focus on Gpx4 in AgRP and POMC neurons was driven by the following rationale: a) Gpx4 is expressed in hypothalamic neurons (Cong et al., 2012); b) HFHS feeding (Y. Thaler et al., 2012; Gao et al., 2014) and fatty acid species such as monounsaturated fatty acids (Obici et al., 2002; Kleinridders et al., 2009) or ceramides (Gao et al., 2011) were shown to induce hypothalamic inflammation and metabolic dysfunction; c) AgRP and POMC

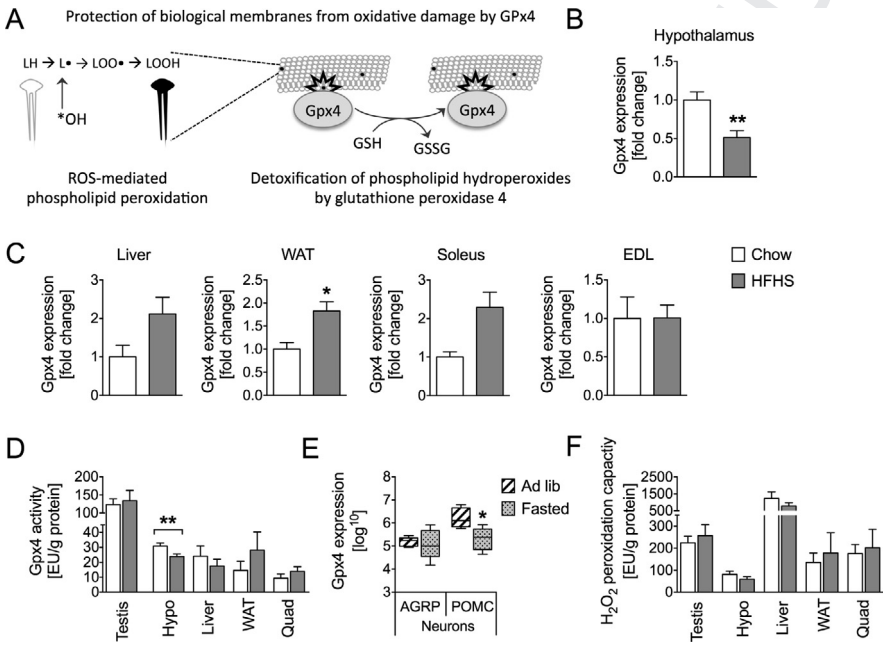

Fig. 1. Diet-induced obesity reduces hypothalamic Gpx4 expression and activity in mice. (A) Schematic representation of Gpx4-mediated membrane protection. Reactive oxygen species (ROS), especially hydroxyl radicals (OH*), induce the formation of phospholipid hydroperoxides (LH – > LOOH), which are detoxified by Gpx4 at the expense of glutathione (GSH). Expression levels of Gpx4 in the hypothalamus (B) and peripheral tissues (C) of male C57Bl/6J mice exposed to chow or HFHS for 17 weeks (Chow: n = 4–6; HFHS: n = 15–18). (D) Enzymatic activity of Gpx4 in the hypothalamus and peripheral tissues of male C57Bl/6J mice exposed to chow or HFHS for 17 weeks (n = 4). (E) Logarithmic expression levels for Gpx4 in AgRP and POMC neurons of young male chow-fed C57Bl/6J mice exposed to ad lib feeding or 24 h of fasting (n = 5–6). Data were generated by Henry et al. (Henry et al., 2015) and deposited under GSE68177. (F) Enzymatic peroxidation capacity of H_2O_2 in the hypothalamus and peripheral tissues of male C57Bl/6J mice exposed to chow or HFHS for 17 weeks (n = 4). Means ± SEM (b-d) or Box and Whiskers with 5–95% percentile (e). $^{*}p < 0.05$, $^{**}p < 0.01$.