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Original article

Acute mental stress induces mitochondrial bioenergetic crisis and hyper-fission along with aberrant mitophagy in the gut mucosa in rodent model of stress-related mucosal disease

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ABSTRACT

Psychological stress, depression and anxiety lead to multiple organ dysfunctions wherein stress-related mucosal disease (SRMD) is common to people experiencing stress and also occur as a side effect in patients admitted to intensive care units; however the underlying molecular aetiology is still obscure. We report that in rat-SRMD model, cold restraint-stress severely damaged gut mitochondrial functions to generate superoxide anion ($O_2^{\bullet-}$), depleted ATP and shifted mitochondrial fission-fusion dynamics towards enhanced fission to induce mucosal injury. Activation of mitophagy to clear damaged and fragmented mitochondria was evident from mitochondrial translocation of Parkin and PINK1 along with enhanced mitochondrial proteome ubiquitination, depletion of mitochondrial DNA copy number and TOM 20. However, excess and sustained accumulation of $O_2^{\bullet-}$-generating defective mitochondria overpowered the mitophagic machinery, ultimately triggering Bax-dependent apoptosis and NF-κB-intervened pro-inflammatory mucosal injury. We further observed that stress-induced enhanced serum corticosterone stimulated mitochondrial recruitment of glucocorticoid receptor (GR), which contributed to gut mitochondrial dysfunctions as documented from reduced ETC complex 1 activity, mitochondrial $O_2^{\bullet-}$ accumulation, depolarization and hyper-fission. GR-antagonism by RU486 or specific scavenging of mitochondrial $O_2^{\bullet-}$ by a mitochondrially targeted antioxidant mitoTEMPO ameliorated stress-induced mucosal damage. Gut mitopathology and mucosal injury were also averted when the perception of mental stress was blocked by pre-treatment with a sedative or antipsychotic. Altogether, we suggest the role of mitochondrial GR-$O_2^{\bullet-}$-fission cohort in brain-mitochondria cross-talk during acute mental stress and advocate the utilization of this pathway as a potential target to prevent mitochondrial unrest and gastropathy bypassing central nervous system.

1. Introduction

Sustained mental ailments like anxiety and depression significantly affect our health by altering physiological homeostasis [1–3]. Stress-related mucosal disease (SRMD) is one such manifestation documented worldwide in patients experiencing stress [4]. Moderate to acute mucosal bleeding in critically ill patients of Intensive Care Unit (ICU) is one of the critical stress-associated phenomena and the mortality rate is significantly high (40–50%) [5,6]. Stomach houses a semi-autonomous nervous system (enteric nervous system, ENS) consisting of five hundred million nerves in the lining of the human gut. It is also the source and/or the depository of many neurotransmitters. ENS is sometimes called the “second brain,” and it arises from the same tissue as the central nervous system (CNS) during development. CNS and ENS continue to influence each other lifelong and interestingly the stress response is manifested through ENS more promptly than any other organ leading to functional gastrointestinal disorders, mucosal bleeding, inflammation, pain, and other bowel symptoms. On the other hand poor gut health has been implicated in various psychophysical disorders [7].

Being the cellular powerhouse, mitochondrial health, biogenesis and protein quality control are matters of critical concern; imbalance of mitochondrial metabolism is associated with oxidative stress and various cytopathologies [8,9]. Mitochondrial structural dynamics [10] is delicately tuned with outer environment. While mild stress induces organellar hyperfusion, moderate to acute stress evokes fragmentation followed by mitophagy in eukaryotes [11,12]. Several quality control proteases like PINK1, PARL and Parkin constantly monitor mitochondrial integrity and ensure timely clearance of damaged organelles [13,14]. Apart from its roles in mitophagy, PINK1 also participates in

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apoptosis regulation [15] while controlling mitochondrial protein turnover and suppressing mtDNA damage [16,17]. Interestingly, severe stress pertaining to irreparable cellular damage triggers apoptosis [18].

In the present study, to mimic an intensive care like ambience of stress in the lab we adapted a model of rodent cold restraint stress [19], which causes gastric mucosal injury and bleeding. Here we report that mental stress induces mitochondrial pathology and shifts mitochondrial structural dynamic balance towards excess fission to damage the gastric mucosa by promoting tissue inflammation and apoptosis. Interestingly, trans-activated hypothalamic-pituitary-adrenal (HPA) axis actively regulates the aforesaid events. Elevated circulating corticosterone with concurrent recruitment of glucocorticoid receptor (GR) in mitochondria switches on a vicious cycle of enhanced mitochondrial fission-mitophagy-bioenergetic crisis, which ultimately triggers cell death to ensure tissue injury during stress.

2. Results

2.1. Acute mental stress prevents ATP formation by affecting electron transport chain, induces mitochondrial pathology and severe fragmentation in gastric mucosa in rodent model of stress-related mucosal disease (SRMD)

The exposure of rats to cold-restraint stress developed significant ($P < 0.001$) gastric mucosal erosions and bleeding whereas control animals did not show any gastric bleeding (Fig. 1A). Time-course studies revealed that stress-exposed animals showed highest injury at 3.5 h (Fig. S1). This time point was chosen for the rest of the experiments unless mentioned otherwise. Interestingly, impairment of stress perception by pre-treatment with diazepam (typical sedative) drastically reduced gastric mucosal injury and bleeding. Furthermore, antipsychotic drug, olanzapine that only interfered with the perception of stress, while keeping the animals alert, offered even better protection under same experimental setup (Fig. 1A). It is worth mentioning that none of the aforesaid drugs offered any protection against indomethacin (a typical NSAID)-induced gastric injury, where brain is apparently not involved (Fig. S2A). Stress-induced gastric mucosal bleeding was evaluated by measuring haemoglobin release in mucosal washing in different experimental sets (Fig. 1A, Fig. S2B).

Next, in order to check whether the manifestation of mucosal damage in stress involved any underlying bioenergetic crisis, we monitored mitochondrial function. Gastric mucosal ATP content was significantly reduced ($P < 0.05$) in rats exposed to stress compared to control (Fig. 1B). Mitochondrial respiration (measured by respiratory control ratio, RCR) and ETC complex I activity were also compromised in the gastric mucosa of rats under stress (Fig. 1C, Fig. S3A). Next, in order to check mitochondrial functional integrity during stress, we evaluated the status of mitochondrial fatty acid oxidation and dehydrogenase activities. Data revealed significantly reduced ($P < 0.01$) fatty acid oxidation (Fig. 1D) and dehydrogenase activity (Fig. S3B) in stress. In addition, significant ($P < 0.01$) depolarization of $\Delta\Psi m$ further confirmed mitochondrial pathology during stress (Fig. S3C). Depolarised mitochondria with compromised ETC complex I generate $O_2^{\bullet -}$ [20] that serves as a progenitor molecule for various other reactive oxidants [21] ultimately causing damage by macromolecular peroxidation and carbonylation [22]. Therefore, we directly measured mitochondrial reactive oxidants accumulation in gastric mucosal cells from “control” and stressed rats. Data indicated heavy accumulation of mitochondrial $O_2^{\bullet -}$ during stress (Fig. 1E). Further, DCFDA-based flow cytometric analysis was performed wherein cells isolated from stressed-rat stomach exhibited high DCFDA-fluorescence revealing the probable occurrence of Fe-catalysed redox reactions (Fig. S4A). A direct impact of elevated mitochondrial reactive pro-oxidants (during stress) was observed from significant decrease in cardiolipin (Fig. 1F) and other biomarkers of oxidative stress [22] including mitochondrial macromolecule oxidation and protein carbonylation (Fig. S4B and C).

Next, to check the probable effects of functional derangement on mitochondrial structure [23] during stress, we followed the expression and localisation of mitochondrial dynamics-associated proteins in the gastric mucosa from control and stressed rats. Confocal microscopic studies documented enhanced expression as well as elevated mitochondrial translocation of the predominant fission mediator Drp1 (green) in the gastric mucosa of stressed rats compared to control (Fig. 1G). Immunoblots further confirmed enhanced Drp1 expression as well as increased mitochondrial translocation during stress (Fig. 1H and I). Moreover, upregulated Drp1 expression was concurrent with enhanced phosphorylation at its serine616 residue (p-Drp-S616) (Fig. 1H) and reduced phosphorylation at serine637 residue (p-Drp-S637) further implying Drp-1-dependent mitochondrial fragmentation during stress (Fig. 1H). Significant changes in the fusion mediators like Mfn 1 and 2 were however indiscernible (Fig. 1H).

2.2. Activation of mitophagy turns insufficient to control the mitochondrial pathology deciding the fate of gastric mucosal cells to undergo apoptosis

To check the plausible elimination of damaged mitochondria we next evaluated mitochondrial content in mucosal tissue by measuring the level of mitochondrial marker TOM 20. Interestingly, it was found to be lower in stress compared to control indicating mitophagic clearance (Fig. 2A). Moreover evaluation of mitochondrial DNA (mtDNA) copy number revealed depletion of mitochondrial content in gastric mucosa of rats exposed to stress (Fig. 2B) and significant induction of mitophagic cascade was evident from Parkin upregulation (Fig. 2C). Further higher magnification documented a considerable enhancement of mitochondrial localisation of Parkin during “stress” (Fig. 2C). To get a deeper insight into the kinetics of mitochondrial remodelling, we evaluated the mitochondrial translocation of key fission-mitophagy markers in a time resolved manner following induction of stress (Fig. 2D). Immunoblot data revealed that mitochondrial translocation of Drp1 initiated early within 30 min of stress induction. Within 1 h of stress initiation, mitochondrial inner membrane potential sensor PARL started degradation, leading to stabilisation of PINK1 on mitochondrial outer membrane. This was succeeded by increase in mitochondrial translocation of Parkin, which peaked at 2 h after stress exposure. Ultimately, ubiquitination of the damaged mitochondria started and eventually peaked at 3.5th h of stress. Moreover, mitochondrial sequestration, that started 1 h after stress exposure, gradually increased and peaked at 165 min (2.75 h) as evident from upregulation of p62 (sequestosome 1) in the mitochondrial fraction.

To check the fate of mucosal cells that was decided by mitochondrial fission and mitophagy, we next investigated the extent of mucosal cell death by following caspase activity in a time resolved fashion following induction of stress. Data indicated a temporal increase in caspase 9 (Fig. 2E) followed by caspase 3 activities in the gastric mucosa (Fig. 2F). Kinetic assessment of mitophagy revealed that mitophagic clearance of damaged mitochondria followed a regressive pattern at the later hours of stress as evident from reduced mitochondrial Parkin recruitment and rate of mitochondrial protein ubiquitination. Comparative kinetic analysis of mitophagy and apoptosis (Fig. 2G-H) therefore clearly indicated the failure of mitochondrial quality control machinery to eliminate damaged organelles and consequent caspase activation in the later phase of the stress. Further, we also observed mitochondrial translocation of Bax (Fig. S5A) along with a significant increase ($P < 0.01$) in cytochrome C in the cytosolic fractions of gastric mucosa isolated from stressed rats (Fig. S5B and C) compared to control. Altered cellular redox status differentially modulates NF-κB signalling and tissue integrity [24,25]. In corroboration with previous reports of NF-κB activation and proinflammatory tissue damage [26], we found that stress-induced mitochondrial pathology was concurrent with NF-κB activation. Immunoblots revealed a significant elevation and nuclear translocation of NF-κB p65 during stress (Fig. 3A). Higher level of IκBα in the control rats (Fig. 3A) further implied its degradation during stress. Interestingly, stress-induced IκBα degradation paralleled with