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Editorial

Modulation of ASIC1a by reactive oxygen species through JFK signaling

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Introduction

Diatomic oxygen gas (O_2) is a vital component of biological systems that contains unpaired electrons in the outer orbital [1]. With this electron configuration, cellular O_2 metabolic processes easily form reactive oxygen species (ROS). ROS is an umbrella term coined to describe the highly reactive chemical byproducts of normal oxygen metabolism such as superoxide anion (O_2^-), hydroxyl radical ($OH^·$), and hydrogen peroxide (H_2O_2) [2-4]. Generated by the mitochondria, O_2^- is the primary ROS produced in the body through oxygen reduction in the electron transport chain (ETC) and Krebs cycle [5]. Through the Haber-Weiss reaction, O_2^- reacts with the non-radical molecule H_2O_2 and generate the highly potent $OH^·$ [6, 7]. At physiological concentrations, ROS plays important roles in maintaining homeostasis through oxidation-reduction processes, metabolic regulation, and cellular adaptation [8]. However, ROS also has a dual role in irreversibly damaging DNA under conditions of oxidative stress. These conditions stem from the overproduction of ROS, leading to an imbalance between free radicals and antioxidants [9]. To prevent oxidative stress, the body contains defense mechanisms, such as the antioxidant system, that suppress ROS activity by attacking harmful macromolecules [10, 11]. Oxidative stress is closely related to the development of neuropathological conditions such as Parkinson's disease, Alzheimer's disease, and stroke [12].

Acidic pH in the body can arise due to varying etiologies. The accumulation of non-oxidized acids, e.g., lactic acid production from anaerobic glucose metabolism, is a common cause of acidosis. The buildup of intracellular protons from ATP hydrolysis also drives the acidification of the cell [13, 14]. Regardless of etiology, studies have demonstrated that acidosis can activate a family of ligand-gated ion channels named acid-sensing ion channels (ASICs), which are highly expressed in the nervous system [15-17]. ASICs have a wide variety of electrophysiological and pharmacological properties depending on their composition of either homotrimeric or heterotrimeric subunits [18, 19]. When activated, the influx of Na^+ ions largely induces neuronal depolarization and generates action potentials [20]. Though it is uncertain that oxidative stress can directly lead to the activation of ASICs, tissue acidosis is a common etiology of disorders that overlap with conditions from oxidative stress as well as inflammation, cancer, and infections [21]. The regulation of ASICs, particularly ASIC1a, by ROS is being investigated further to provide more knowledge on the pathogenesis of neurodegenerative diseases. In fact, a particular study examined the upregulation of ASIC1a by H_2O_2 through a family of protein kinases, the c-Jun N-terminal kinase (JNKs) signaling pathway [22]. JNKs are responsible for regulating various processes such as cell differentiation and proliferation, inflammatory responses, and expression of proteins [23]. Regulators of the JNK pathway are encoded by three genes: JNK1, JNK2, and JNK3, which potentially contribute to insulin resistance and cell signaling [24]. Deregulation of JNK can lead to several consequences involving neurodegenerative disease, cardiac hypertrophy, autoimmune disease, and cancer [25]. Thus, it is

important to identify regulators and effectors of the JNK signaling pathway. Thus, it is important to identify regulators and effectors of the JNK signaling pathway. In this commentary, we discussed the molecular mechanisms of oxidative stress on ASIC1a expression.

Upregulation of ASIC1a by hydrogen peroxide through the JNK pathway

A recent study published in the *Acta Pharmacologica Sinica* investigated the effects of H_2O_2 on ASIC1a expression in neuronal cell lines containing ASICs and primary cultured mouse cortical neurons by using a combination of techniques including cell culture, western blotting, lactate dehydrogenase assay, and patch-clamp recording [22]. They found that concentrations of H_2O_2 greater than 40 μM significantly increased NS20Y cell injury after 24 hours; however, concentrations of 20 μM or lower did not reveal clear cell injury. Exposure to 20 μM of H_2O_2 revealed increased ASIC1a expression as compared to the control level in NS20Y cells. Following exposure to H_2O_2 for 6 hours, ASIC1a expression increased 1.50 ± 0.08-fold in NS20Y cells. This increase in ASIC1a expression was not observed when NS20Y cells were exposed to H_2O_2 for 3 hours. Further investigation was done to confirm the effects of H_2O_2 on ASIC1a currents by using whole-cell patch-clamp recordings. The peak amplitude of ASIC currents was increased when NS20Y cells expressing ASIC1a were pretreated with 20 μM H_2O_2 for 24 hours. Additionally, the current density of ASIC1a was also increased from -20.38 ± 3.16 to -34.92 ± 4.08 pA/pF, which indicated an increase in functional channels and protein expression. To determine if H_2O_2 had a direct modulatory effect on ASIC1a, cells were perfused with 20 μM H_2O_2 in both normal pH solution (pH 7.4) and acidic pH solution (pH 6.0) for 5 minutes. The results showed no significant change in amplitude of the currents, indicating that there was no difference between the two pH conditions under short-term conditions in this model.

To further explore the mechanisms underlying the effects of H_2O_2 on ASIC1a, activations of mitogen-activated protein kinase (MAPK) signaling pathways (including ERK, JNK, and p38 pathways) were investigated. JNK, p38, and ERK1/2 pathways were dramatically upregulated in NS20Y cells expressing ASIC1a after being treated with 20 μM H_2O_2 within 30-60 minutes. Next, the effects of MAPK pathway inhibitors on ASIC1a were studied to determine which specific pathway is involved in H_2O_2-induced changes in ASICs. Introducing 10 μM of U0126 or SB203580, inhibitors of the MEK/ERK pathway and p38 MAPK pathway respectively, did not affect ASIC1a expression, suggesting that these pathways are not involved in H_2O_2 induced changes. However, introducing 10 μM of SP600125, an inhibitor of the JNK pathway, abolished the H_2O_2-mediated increase of ASIC1a expression and decreased basal ASIC1a expression. Additionally, SP600125 led to decreased ASIC1a expression to 0.36 ± 0.08 and 0.30 ± 0.07 regardless of the presence of H_2O_2. Furthermore, surface expression of ASIC1a by H_2O_2 was inhibited in the presence of SP600125, which was similar to the results of overall protein expression. They also examined the changes in ASIC current density when combined with SP600125 for 24 h, and the results suggested that the current density dramatically decreased and completely abolished the H_2O_2-mediated increase in ASIC current density. When NS20Y cells were treated with 10 μM SP600125, ASIC currents were not inhibited after 5 minutes, suggesting that SP600125 does not directly affect ASIC gating, but rather affects ASIC protein expression through long-term treatment.

The study also examined the effects of H_2O_2 on ASIC1a in primary cultured mouse cortical neurons. The findings were similar to those in NS20Y cells, with significant neuronal injury resulting from high concentrations of H_2O_2 (40 and 80 μM). Lower concentrations of H_2O_2 (less than 20 μM) did not cause injury. In mouse cortical neurons treated with 20 μM H_2O_2, ASIC1a expression increased 1.48-fold and the density of ASIC currents increased 1.41-fold when compared with the control. The ASIC2 gene, an ASIC subunit, was also studied to determine its interactions with H_2O_2. The results suggested that H_2O_2 did not affect ASIC2 after 24 hours.

Lastly, the study compared the effects of PcTx1 blockade on ASIC pretreated with and without H_2O_2. The results confirmed that H_2O_2 increases the sensitivity of ASIC currents to the blockade, with 34.20% ± 5.95% of the ASIC currents inhibited by PcTx1 in control cells and 56.27%

± 5.61% of ASIC currents inhibited in H_2O_2-treated cells. These results indicate that more ASIC1a channels are expressed and distributed on the plasma membrane when treated with H_2O_2, reaffirming that H_2O_2 upregulates functional ASIC1a expression.

Perspective

The findings of the study ultimately provide evidence that H_2O_2 upregulates the expression and increases the function of ASIC1a through the JNK signaling pathway. However, the independent roles of oxidative stress and ASIC1a in the development of neurodegenerative diseases have not been well-studied to determine an effect on each other. Specifically, the literature regarding the effect of oxidative stress on the expression of ASIC1a is sparse, with the present study being the first to evaluate the effects of physiological concentrations of H_2O_2 on ASIC1a expression. In a previous study, Zha et al. found that exposure to higher, non-physiological concentrations of H_2O_2 (1-10 mM) inhibited the surface expression of ASIC1a by increasing the formation of intersubunit disulfide bonds [26]. The normal range of H_2O_2 in human blood has been suggested to be between 1 and 5 μM, with increased concentrations in many chronic disease conditions [27]. Wu et al. used 20 μM H_2O_2 as a pathologically relevant concentration to simulate a disease state [22]. Thus, the differences in findings between the studies are due to the varying concentrations used. A recent study by Verkest et al. combined both *in vitro* and *in vivo* approaches that demonstrate that JNK is a post-translational phosphorylative regulator of rodent and human ASIC1b and ASIC3 channels. Their findings suggest that JNK can lead to peripheral sensitization and pain hypersensitivity [28]. A prospective pain management therapy can target the JNK signaling pathway to prevent oxidative stress from potentiating ASIC expression. Numerous malignancies, including melanoma, breast cancer, and colorectal cancer, have been linked to the JNK pathway and show enhanced signaling activity [29]. Additionally, neurodegenerative conditions such as Alzheimer's disease, multiple sclerosis, and Parkinson's disease also exhibit overactivation of JNK signaling [30-32]. Hence, potential therapies can target oxidative stress to downregulate the JNK signaling pathway and prevent tumorigenesis and neurodegenerative diseases. Further investigation of the inhibition of oxidative stress and ASIC expression and its direct outcome on tumorigenesis and neurodegenerative diseases is warranted.

Although MEK/ERK and p38 kinase pathways were found to have no involvement in H_2O_2-induced changes in ASICs, a recent study by Peng et al. found that ASIC currents were potentiated by hydrogen sulfide (H_2S) in a time- and concentration-dependent manner through the MAPK-Erk1/2 signaling pathway [33]. The observed potentiation was induced through an increased expression of ASIC1a at the plasma membrane. Notably, studies have identified interactions between H_2S, ROS, and NO in their production and downstream signaling, with H_2S being found to protect the body from oxidative stress [34-36]. This suggests that H_2S has dual mechanisms to potentiate ASIC currents and reduce the concentrations of ROS and RNS [30, 33]. The usage of H_2S as a therapy related to oxidative stress and ERK1/2-related pathologies requires further research. In rat dorsal root ganglion neurons, brief exposure to the proinflammatory cytokine tumor necrosis factor-α (TNF-α) was found to rapidly increase ASIC-mediated functional activity via the p38 pathway [37]. Prospective therapies that target TNF-α and/or the p38 pathway may downregulate ASIC activity and therefore treat neurodegenerative diseass that result from oxidative stress. Future studies should be performed to expand our knowledge of the possible regulators of ASIC activity via mechanisms of the MAPK signaling pathways.

Along with future investigations on the effects of other ROS, the effects of reactive nitrogen species (RNS) including NO and peroxynitrite ($ONOO^-$) on ASICs should be evaluated. Previous studies have suggested that NO potentiates the effects of ASICs, with a potential external effect through the oxidation of cysteine residues [38]. A study performed by Jetti et al. determined the role of NO in ASIC-mediated cell death and found that NO promoted cell death by potentiating ASICs during mild and moderate acidosis, though this effect subsided in the presence of severe acidosis with the inhibition of NO synthase [36]. Because the current literature regarding this topic is limited, our knowledge of the molecular mechanisms behind the impact of RNS on ASICs may be

significantly aided by further research of their relationship.

In addition, research should be performed on other antioxidants to further study their potential effects on ASIC1a expression and channel function. Prior studies have demonstrated the potentiation of ASIC currents by reducing agents dithiothreitol (DTT) and glutathione [39], though further investigation is required to gain a more robust understanding of the molecular interactions between antioxidants and ASIC expression, and whether more antioxidants such as glutathione and vitamin E play a role in ASICs.

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Disclosure of conflict of interest

None.

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