Oxidative stress and anxiety

Relationship and cellular pathways

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High O₂ consumption, modest antioxidant defenses and a lipid-rich constitution make the brain highly vulnerable to redox imbalances. Oxidative damage in the brain causes nervous system impairment. Recently, oxidative stress has also been implicated in depression, anxiety disorders and high anxiety levels. The findings which establish a link between oxidative stress and pathological anxiety have inspired a number of other recent studies focusing on the link between oxidative status and normal anxiety and also on a possible causal relationship between cellular oxidative stress and emotional stress. This review examines the recent discoveries made on the link between oxidative status and normal anxiety levels and the putative role of oxidative stress in genesis of anxiety. We discuss the different opinions and questions that exist in the field and review the methodological approaches that are being used to determine a causal relationship between oxidative and emotional stress.

Introduction

Anxiety is an aversive emotional state, in which the feeling of fear is disproportionate to the nature of the threat.¹ In response to threatening situations, the feeling of the emotion that constitutes the subjective feature of anxiety is accompanied by emotional stress, which involves behavioral, expressive and physiological features, such as an avoidance of the source of the danger, assuming defensive postures and an increase in blood pressure, respectively.¹,² Anxiety is a normal emotional response to a threat or potential threat. When this emotion is inappropriate, extreme and persistent, it is classified as pathological.¹,³ Anxiety is implicated in a number of psychiatric disorders, such as depression, panic attacks, phobias, generalized anxiety disorder, obsessive-compulsive disorder and post-traumatic stress disorder.³ Anxiety disorders affect approximately 28.8% of the US population,⁴ imposing both an individual and a social burden that amounts to a total cost of $42.3 billion in the US in 1990.⁵ Anxiety disorders are the most common class of psychiatric disorders in the US⁴ and many other countries.⁶ According to an ESEMeD study including six European countries, the 12-month prevalence of inappropriate anxiety was 6.4%.⁷ In the most recent systematic review of studies conducted in 16 European countries, however, this value was estimated to be 12%.⁸ In an obese population in the UK, 56% of patients met the minimum criteria for an anxiety disorder.⁹ It is estimated that one-eighth of the total population worldwide suffers from inappropriate anxiety.¹⁰ Population-based studies have shown that anxiety disorders frequently go untreated.¹¹,¹² Predominantly, the research that has been performed on anxiety has focused on the regulatory systems, including gamma-aminobutyric acidergic (GABAergic) and serotoninergic systems among others. However, Kuloglu et al.¹³,¹⁴ recently established a link between oxidative stress and certain anxiety disorders (obsessive-compulsive disorder and panic disorder), demonstrating that other systems, such as oxidative metabolism, can affect the regulation of anxiety. These findings, which establish a link between oxidative stress and pathological anxiety, inspired a number of other recent studies focusing on the link between oxidative status and normal anxiety (Table 1) and also on a possible causal relationship between cellular oxidative stress and emotional stress.

It is well known that low/moderate concentrations of reactive oxygen species (ROS) affect a great number of physiological functions.¹⁵ However, when ROS concentration exceeds the antioxidant capacity of an organism, animal cells enter a state termed oxidative stress, in which the excess ROS induces oxidative damage to cellular components.¹⁵,¹⁶ As a result, oxidative stress has been implicated in a large range of diseases, including cancer, diabetes, male infertility, autoimmune diseases, atherosclerosis and cardiovascular disorders.¹⁵-¹⁷

The brain is highly vulnerable to oxidative stress due to its high O₂ consumption, its modest antioxidant defenses and its lipid-rich constitution.¹⁸,¹⁹ Human brain utilizes 20% of oxygen consumed by the body even though this organ constitutes only about 2% of the body weight.¹⁹,²⁰ When the production of oxygen-derived metabolites prevails over the brain defense systems, however, oxidative damage to nucleic acids, proteins and neuronal membrane
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Table 1 Data establishing the link between pathological/normal anxiety and oxidative cell pathways and mechanisms

| Subjects | Expression of antioxidant genes | Activity of antioxidant proteins | Lipid peroxidation markers | Direct evaluation of oxygen-derived species |
|----------|---------------------------------|---------------------------------|---------------------------|----------------------------------------|
| Pathological anxiety | Patients with obsessive-compulsive disorder and panic disorder | | | Indirect evaluation of oxygen-derived species |
|          | (Kuloglu et al.13,14) | In erythrocytes: Superoxide dismutase*, catalase, glutathione peroxidase* | In plasma: Malondialdehyde* | In brain: Glyoxalase 1* and glutathione reductase 1* |
| Normal trait-anxiety | Mouse strains with high-related phenotypes (Hovatta et al.25) | In brain: Glyoxalase 1* and glutathione reductase 1* | | In brain and peripheral cells: Neurones*, glial cells*, granulocytes*, monocytes* and lymphocytes* |
|          | Anxious Vs. non-anxious Swiss albino mice (Rammal et al.41,42) | | | |

*significantly different from controls (healthy humans, strains with low-anxiety-related phenotypes, non-anxious mice).

Lipids, which are rich in highly polyunsaturated fatty acids, can occur. In presence of oxidative stress, the lipid-rich constitution of brain favors lipid peroxidation that results in decrease in membrane fluidity and damage in membrane proteins inactivating receptors, enzymes and ion channels. As a result, oxidative stress can alter neurotransmission, neuronal function and overall brain activity. Oxidative stress has been associated with several diseases which are specific for nervous system impairment including neurodegenerative diseases and neuropsychiatric diseases, such as schizophrenia and major depressive disorder. The intrinsic oxidative vulnerability of the brain has led some authors to suggest that oxidative damage may be a plausible pathogenic factor for certain neurological diseases including neuropsychiatric disorders.

In this review, we discuss the relationship between oxidative stress and normal anxiety by presenting the recent advances in the field and the different views that exist. We also review the methodological approach for determining the causal relationship between oxidative stress and emotional stress.

A Link Between Oxidative Stress Metabolic Pathways and Anxiety-Related Phenotypes

In 2005, Hovatta et al. obtained surprising results in a genetic manipulation using lentivirus-mediated gene transfer: local overexpression of glutathione reductase 1 and glyoxalase 1 in the cingulated cortex of the murine brain results in an increase of anxiety-like behavior, while inhibition of glyoxalase 1 expression produces low-anxiety mice. Thus, Hovatta et al. were able to make a causal link between the antioxidative status of the brain and anxiety-related behavior and hypothesize that glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. It is worth mentioning that in vivo, antioxidant genes are overexpressed in response to an uncontrolled production of ROS. Indeed, in vivo, excessive ROS accumulation induces the overexpression of the glutathione redox system, including glutathione reductase, and a general overexpression of endogenous antioxidants. In the
trait anxiety. These results are discordant with those of Hovatta et al., however, and complicate the understanding of the relationship between oxidative stress and trait anxiety. Krömer et al. and Ditzen et al. examined the expression of glyoxalase 1 in several areas of the brain and in red blood cells and found that this protein is expressed more in a line with a low-anxiety-related behavioral phenotype than in a line with a high-anxiety-related behavioral phenotype. It is worth noting that Hovatta et al.suggested that there is a link between glyoxalase 1 and oxidative stress but that this link is indirect. Indeed, glyoxalase 1 is an enzyme of the glyoxalase system, which protects against carbonyl stress; glutathione is a determinant cofactor for the enzymatic reaction that is catalyzed by glyoxalase 1.

A Link Between Oxidative Stress Metabolic Pathways and Anxiety-Related Behavior

The disagreements in the data of Hovatta et al., Krömer et al. and Ditzen et al. can be partly explained by the differences in the genotypes of the strains. Thus, it would be interesting to compare in the same strain the oxidative status of mice with contrasting levels of anxiety, rather than compare the oxidative status of strains that differ in their anxiety-related phenotypes. We have observed that naive Swiss albino male mice have a large heterogeneity in their anxiety levels. In first order regression analyses of the performances of mice in the light/dark choice test, which are used as a behavioral indices of anxiety, and the intracellular ROS accumulation in blood granulocytes, we found correlation coefficients (R²) ranging from 0.61 (p < 0.01) to 0.73 (p < 0.001) (Fig. 1).

Levels of ROS can be evaluated directly with sensors, such as 2',7'-dichlorofluorescin diacetate (DCFH-DA), or indirectly by measuring the levels of certain antioxidant enzymes, byproducts of lipid peroxidation or some transition metals, such as copper, zinc and iron. In our study, the intracellular redox status of the cells was evaluated using the tracer DCFH-DA, a well known sensor of ROS. To confirm the relationship between oxidative stress and emotional stress, we comparatively evaluated the peripheral oxidative status of mice with contrasting levels of anxiety (anxious and non-anxious). Mice with intermediate behaviors were eliminated.

During the same period in which the Hovatta et al. study was being conducted, other studies were performed on two Swiss CD1 mouse lines with contrasting anxiety-like behavioral phenotypes. These mice were generated from wild type mice after >15 generations of selection. Results from these studies have led researchers to propose that glyoxalase 1 might be a biological marker for oxidative stress and anxiety: Relationship and cellular pathways
studies38,41,42 are in good concordance with the initial findings of S,R-sulfoximine (BSO), an inducer of oxidative stress, induces behavior in mice. They found that treating mice with buthionine-s,R-sulfoximine (BSO), an inducer of oxidative stress, induces oxidative stress and anxiety. While all of the data demonstrate that oxidative stress and anxiety are linked, there is a lack of a clear causal relationship between these stresses.

In agreement with our recent findings,41,42 Yasunari et al.43 observed a significant relationship between trait anxiety and ROS formation in monocytes of hypertensive individuals. Recently, Masood et al.29 published work that contributes to the understanding of the relationship between oxidative stress and anxiety. They examined the direct effect of oxidative stress on anxiety-like behavior and established that oxidative stress leads to anxiogenic behavior in mice. They found that treating mice with buthionine-S,R-sulfoximine (BSO), an inducer of oxidative stress, induces anxious behavior through the NADPH oxidase pathway. The anxiogenic behavior due to BSO treatment was observed in several mouse models of anxiety, including elevated plus maze, hole-board and open field tests. Masood et al.29 induced oxidative stress in mice by depleting glutathione with BSO inhibition of gamma-glutamylcysteine synthetase. Glutathione depletion causes a myriad of cellular stresses, including oxidative, nitrosative and carbonyl stresses, as glutathione is an important determinant of the oxygen, nitrogen and dicarbonyl metabolisms.15,16,35,44

Excessive production of ROS induces oxidative damage of cellular structures;15,16,19,45 production of reactive nitrogen species triggers nitrosylation reactions, which can alter the structure of proteins to inhibit their normal function;15,16,46 excessive accumulation of reactive dicarbonyl compounds leads to damage of protein and nucleotides by dicarbonyl glycation.35-37

### Indirect Evidence for the Causal Link Between Oxidative Stress and Anxiety

Recent data from Desrumaux et al.47, Souza et al.48 and Berry et al.49 provide indirect evidence for the causal link between oxidative stress and anxiety-related behavior. Desrumaux et al.47 showed that vitamin E deficiency in the mouse brain significantly increases the levels of central oxidative stress markers, resulting in anxiogenic behavior without abnormalities in the locomotor performance of the mice. Souza et al.48 demonstrated in rats that the consumption of a highly palatable diet enriched with sucrose leads to an obese phenotype, increases protein oxidation in the frontal cortex and induces anxiety-like behavior in the dark/light choice test without altering locomotion in an open field test. Berry et al.49 showed that mice developed anxious behavior during aging, likely due to the accumulation of oxidative damage, which is a characteristic of the aging process in animals.50,51

In addition, Berry et al.49 showed that a deletion of the p66Shc longevity gene in mice, which results in lower levels of oxidative stress and an extended life span, decreases anxiety-related behavior. Overall, the data presented in these reports suggest that oxidative stress can provoke anxious behavior in rodents.

### Conclusion

This review summarizes the data to support a link between oxidative stress and anxiety. While all of the data demonstrate that there is a link between oxidative stress and high-anxiety-related behavior, a cause-effect relationship has yet to be completely established. Some of these studies suggest that oxidative stress causes anxiety-related behaviors but do not explain the underlying mechanisms. While there are some limits in the approach to establish the anxiogenic effect of oxidative stress, the available data are consistent this causal relationship. The potential causal role of oxidative stress on anxiety may generate interest in antioxidants. Masood et al.29 were able to show that oxidative stress-related anxiety can be reversed in mice upon inhibition of NADPH oxidase or phosphodiesterase-2, enzyme that is indirectly implicated in oxidative stress mechanisms. Surprisingly, they found that diazepam, which is a well known anxiolytic, does not fully reverse the oxidative stress-related anxiety. These results point to a possible use for antioxidants...
in the prevention or reduction of high anxiety. Further research will be necessary to show whether anxious subjects need more antioxid-
ants than non-anxious subjects. Recent work has shown that some dietary polyphenols have both anxiolytic and antioxidant effects, which may be beneficial to anxious subjects.

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