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Dityrosine administration induces novel object recognition deficits in young adulthood mice

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HIGHLIGHTS

- Intragastric administration of dityrosine impairs mice memory in novel object recognition task.
- Dityrosine administration does not impair the spatial learning and memory of mice in Morris water maze.
- Neurobehavioral function such as locomotor activity, anxiety and social interaction are not affected by dityrosine.
- Dityrosine administration declines hippocampal relative mRNA expression of NMDA receptor subunits *Nr1*, *Nr2a*, *Nr2b*; *Bdnf*, *Trkb*.

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ABSTRACT

Dietary modifications have been shown to contribute to the physical and mental diseases. Oxidative modifications of protein can be easily found in protein-rich food such as meat and milk products. Previous studies mainly focus on the consequences of lipid oxidation products intake in vivo, but the effects of protein oxidation products consumption have been largely neglected. Oxidants have been shown to play an important role in aging and neurodegenerative diseases. Dityrosine is the oxidated product of tyrosine residues in protein which is considered as a biomarker for oxidative stress, but the potential deleterious effects of dityrosine are unknown. In the present study, we explored the effects of dityrosine administration on the behavioral aspect. We found that dityrosine-ingested mice displayed impaired memory during novel object recognition test, but no influence to the spatial memory in Morris water maze compared with the saline group. Other aspects of neurobehavioral function such as locomotor activity, anxiety and social behavior were not affected by dityrosine ingestion. Furthermore, we found that dityrosine-ingested mice showed decreased expression level of NMDA receptor subunits *Nr1*, *Nr2a*, *Nr2b* as well as *Bdnf*, *Trkb*. Our study suggests that dityrosine exposure impairs hippocampus-dependent nonspatial memory accompanied by modulation of NMDA receptor subunits and *Bdnf* expression.

1. Introduction

In general, food processing improves taste of food, and prolongs preservation. However, heat treatment, irradiation, light exposure and oxygen in processing will potentially lead to a decrease of product quality and nutritional value by oxidative modification to vitamins, lipids as well as proteins. Lipid oxidation is one of the major degradative processes responsible for changes in flavor, color, and texture. The oxidation of unsaturated lipids results in generation of cytotoxic and genotoxic compounds [1,2]. Consumption of oxidized food components will trigger increased oxidative stress in living tissue which will contribute to some diseases [3]. Dietary lipid oxidation products like 4-Hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) have been demonstrated to be risk factors to human health [4], such as cardiovascular diseases [5]. Another important target of oxidative damage is food proteins. After discovering that myofibril protein was affected by ROS during meat maturation and storage [6], increasing number of researchers started to focus on the occurrence of protein oxidation in muscle foods [7–9] and milk products [10,11].

Protein oxidation could be induced directly by reactive oxygen species (ROS) and reactive nitrogen species (RNS) or indirectly by secondary products of oxidative stress, thus leading to modification of amino acid side chains and fragmentation, aggregation and polymerization of protein. Depending on the targets and the oxidizing agents, the consequences of protein oxidation include loss of sulfhydryl groups, formation of protein carbonyls, formation of cross-links, and modification of aromatic amino acids, among others [12]. All above result in loss of nutritional value and functionality of food proteins [9,12,13]. Since protein oxidation products will accumulate during processing and storage, it is crucial to investigate the impact of intake such dietary oxidized proteins

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on human health. Most studies carried out on protein oxidation during the last several decades have been conducted to examine the oxidized protein as a biomarker in neurodegenerative and other diseases (i.e. Alzheimer's disease, acute myocardial infarctions) [14–17]. But the influence of dietary oxidized proteins on human health has not been fully explored.

In vivo, the quality of protein can be described by its ability to achieve certain metabolic functions which are largely dependent on its constituent amino acids, its peptide sequence, the spatial arrangement in its native structure and its concentration and bioavailability [18]. Oxidative modification of protein is believed to alter protein structure and function, thus, leading to metabolic disorders. Our previous studies have shown that dietary oxidized casein induced hepatic and renal injury in mice by impairing antioxidant defense system and altering the expressions of fibrosis-related genes [19].

Under oxidative stress, protein carbonyls are increased, sulfur-containing amino acids (cysteine and methionine) and aromatic amino acids (tyrosine, phenylalanine, and tryptophan) are the most vulnerable to oxidative modifications. Protein carbonyl derivatives are elicited by directly oxidative attack on Lys, Arg, Pro or Thr, or by secondary reaction of Cys, His or Lys residues with reactive carbonyl compounds [20]. Protein carbonyls are considered to be a broad marker of oxidation. Dalle-Donne reviewed the list of diseases associated with carbonylated protein, including Alzheimer's disease, chronic renal failure, and diabetes [21]. Modification of aromatic amino acids is another way to form oxidized protein, for instance, tryptophan and tyrosine could be modified to kynurenine and dityrosine/3-nitrotyrosine, respectively. Kynurenine is a central metabolite of tryptophan degradation through the kynurenine pathway. Kynurenine and its metabolites have been demonstrated to have neuroactive properties [22,23], and have been described in several neurodegenerative disorders such as Parkinson's disease [24], Huntington's disease [25], schizophrenia [26] and Alzheimer's disease [27]. A recent study reported that administration of kynurenine induced alterations in object recognition test in C57BL/6J mice [28]. Furthermore, kynurenic acid exposure during adolescence has been demonstrated to impair long-term potentiation in adulthood [29]. 3-Nitrotyrosine and dityrosine are considered to be more specific markers of oxidative stress compared to protein carbonyl [30,31]. 3-Nitrotyrosine is the result of nitration of tyrosine residues, which has been demonstrated to be an endogenous contributing factor to neuronal loss, in addition to being a maker of oxidative and nitrative processes [32]. Whereas, dityrosine is formed through inter-molecular cross-linking of two tyrosyl radicals which are generated from interaction of ROS and tyrosine residues [33,34]. Dityrosine has been selected as a biomarker for oxidative stress of protein due to its chemical stability in oxygen, high pH and its highly resistance to acid hydrolysis and proteases. Abundance of researches has revealed increased level of dityrosine in the Alzheimer's disease brain, especially in the hippocampus, which may be related to oxidative damage [14,31,35–37]. However, the implication of dietary dityrosine on brain function is unexplored.

In order to investigate the consequence of dietary dityrosine, we performed an 8-week intragastric administration experiment using C57BL/6J mice, and then conducted a battery of behavioral experiments. Locomotor activity and anxiety-like behavior were assessed in open field and elevated plus maze paradigms. Memory performance was tested in novel object recognition and Morris water maze paradigms. Sociability was examined in a three-chamber social interaction paradigm. Furthermore, we have examined the expressions of hippocampus-dependent learning and memory related genes.

2. Material and methods

2.1. Animals

Four-week-old male C57BL/6 J mice were purchased from the Model Animal Research Center of Nanjing University. All animals were housed under standard laboratory conditions (temperature: 22 ± 1 °C; humidity: 60%; 12 h light/dark cycle) with free access to food and water. Animal care procedures were conducted in accordance with the institution's guideline for the care and use of laboratory animals.

2.2. Treatments

Mice were randomly divided into two groups (ten each). Mice were received either dityrosine (320 μg/kg/day) (DT group) or 0.9% saline solution (saline group). The mice were intragastric administered with dityrosine or saline solution daily between 16:00 and 18:00 for 8 weeks.

A battery of behavioral tests were conducted one week after 8-week intragastric administration in the following order: open field test, novel object recognition test, social interaction test, elevated plus maze and Morris water maze. The timeline of the behavioral tests was shown in Table 1. We used an USB camera controlled by the ANY-maze Software to automatically video track the activities.

2.3. Open field test

Open field test (OFT) was used to measure locomotor activity and/or anxiety-like behavior of mice. The apparatus was a square field (40 × 40 × 30 cm). The testing area was divided into 16 squares (10 × 10 cm) on the computer tracking system. The fours quares located in the central area were defined as the central zone. For assessing open field activity, each animal was placed in the center of the box and allowed to explore freely for 30 min. The parameters measured comprised: total distance traveled, central distance traveled, number of entries into the central zone, and time spent in the central zone.

2.4. Novel object recognition

Non-spatial declarative memory was assessed in novel object recognition test (NORT). Novel object recognition experiments were conducted as described previously [38]. The day after open field test, habituation session was performed. During the habituation session, mice were placed into the open field box without objects for 10 min. Then a training session and a test session were conducted. On the training day, mice were placed in the open field box with two identical objects which were either hard plastic cylinders (ϕ: 5 cm; H: 10 cm) or woody rectangular blocks (4 × 4 × 10 cm) for 10 min. Twenty-four hours after the training session, mice were returned to the box and were allowed to explore the two objects, one identical to the original object and the other replaced by a novel object, for 10 min. The mouse was considered to be exploring an object when its nose was within 2 cm of the object. Data such as time spent in exploring the objects were collected in both the training session and the test session. Furthermore, discrimination of the novel object in the test session was calculated using discrimination index (DI) [DI = (novel object exploration time − familiar object exploration time) / total exploration time].

2.5. Social interaction test

A modified three-chamber protocol was used as described [39]. The apparatus are comprised of a rectangular, three-chamber box. Each chamber was 45 × 20 cm and the dividing walls were made from

Table 1
Schedule of the behavioral tests.

Days	Procedure
1	Open field test
2–4	Novel object recognition
12–14	Social interaction test
18	Elevated plus maze
22–27	Morris water maze