Behavioural Brain Research 284 (2015) 125–130

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

The co-occurrence of zinc deficiency and social isolation has the opposite effects on mood compared with either condition alone due to changes in the central norepinephrine system

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HIGHLIGHTS

- Rats were subjected to zinc deficiency (ZD) and/or social isolation (SI).
- Co-occurrence of SI and ZD had anxiolytic and antidepressive effects.
- Norepinephrine transporter (NET) expression was decreased by ZD alone.
- NET expression was decreased further by the co-occurrence of ZD and SI.

ARTICLE INFO

Article history:
Received 7 November 2014
Received in revised form 2 February 2015
Accepted 3 February 2015
Available online 11 February 2015

Keywords:
Zinc deficiency
Social isolation
Elevated plus maze test
Forced swim test
Cerebral norepinephrine concentration
Norepinephrine transporter

ABSTRACT

Nutritional and social environmental problems during the early stages of life are closely associated with the pathophysiology of mood disorders such as depression. Disruption or dysfunction of the central norepinephrine (NE) system is also considered to play a role in mood disorders. Therefore, we evaluated the effects of zinc deficiency and/or social isolation on mood and changes in the central NE system using rats. Compared with the controls, the rats subjected to zinc deficiency or social isolation alone exhibited increased anxiety-related behavior in the elevated plus maze and greater depression-like behavior in the forced swim test. However, the co-occurrence of zinc deficiency and social isolation resulted in decreased anxiety-related behavior and control levels of depression-like behavior. Social isolation alone decreased the rats' cerebral NE concentrations. The expression of the NE transporter was not affected by social isolation alone, but its expression in the locus coeruleus was markedly decreased by the co-occurrence of social isolation and zinc deficiency, and this change was accompanied by an increase in the blood concentration of 3-methoxy-4-hydroxyphenylglycol, which is a marker of central NE system activity. These findings suggest that zinc deficiency or social isolation alone induce anxious or depressive symptoms, but the presence of both conditions has anxiolytic or antidepressive effects. Furthermore, these opposing effects of mood-related behaviors were found to be associated with changes in the central NE system.

1. Introduction

During development, the environment plays a significant role in the pathophysiology of mood disorders such as depression [1,2]. There are many environmental problems that can contribute to mood disorders if they occur during the early stages of life, and nutritional and social environmental problems play particularly important roles in such conditions. Food restriction in adolescence or exposure to social environmental stress in early life can have a profound influence on individuals' susceptibility to depression and other mood disorders in adulthood [1,2]. Therefore, it is reasonable to evaluate the pathophysiology of mood disorders by examining the effects of nutritional and social environmental problems.

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http://dx.doi.org/10.1016/j.bbr.2015.02.005

Various nutritional problems are associated with mood disorders. Recently, zinc deficiency (ZD) has often been reported as a potential cause of mood disorders [3]. Zinc is a metal that is essential for the development and maintenance of brain function [4]. Furthermore, ZD can cause depressive symptoms. Depression is associated with a lower concentration of zinc in the peripheral blood [5], and a significant negative correlation was detected between the blood zinc concentration and depressive symptoms [6]. During imipramine treatment, zinc supplementation results in increased efficacy and a more rapid therapeutic response, particularly in patients who were previously unresponsive to antidepressant pharmacotherapy [7]. Also in rats, ZD was found to increase depressive-like and anxiety-related behavior [8,9].

Social isolation (SI) is one of the most important social environmental problems associated with mood disorders. Children that are exposed to SI were found to be at elevated risk of depression [10], and SI also reduces the efficacy of treatment for depression [11]. In primary care, SI is often used for depression screening [12]. Previous studies using rats have suggested that anxiety-related behaviors can develop in early adulthood if isolation occurs in early pre-adolescence and persists until the middle period of mid-adolescence [13–15]. Similarly, many reports have suggested that SI increases depressive-like behavior [16].

As described above, both ZD and SI can induce a depressive state. Therefore, it was assumed that the co-occurrence of SI and ZD would result in severe mood disorders. However, in a previous preliminary study using rats we found evidence to suggest that although SI or ZD alone increases anxiety-related behavior, the co-occurrence of SI and ZD might decrease it [17]. In this study, we performed further examinations to confirm our findings, and we also evaluated the effects of ZD and/or SI on depressive-like behavior. Norepinephrine (NE) is one of the neurotransmitters associated with mood. Many studies have shown that disruption or dysfunction of the central NE system is involved in mood disorders [18,19]. The NE transporter (NET) is expressed in the presynaptic terminals of NE neurons and plays a critical role in NE reuptake and regulates the synaptic NE concentration [20,21]. The concentration of the NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) is considered to be a marker of central NE system activity [22]. Therefore, in addition to behavioral tests we elucidated the mechanisms responsible for the behavioral changes induced by ZD and/or SI through evaluations of the central NE system; i.e., of brain NE concentrations, NET expression, and blood MHPG concentrations.

2. Materials and methods

2.1. Animals and experimental protocol

All protocols were consistent with the National Institutes of Health policy regarding the use of animals in experimental research. The institutional animal care committee at the University of Fukui approved the experiments. Three-week-old male Wistar rats were purchased from Sankyo Labo Service Corp. (Tokyo, Japan) and were housed individually (SI) or in groups (GP). They were housed in cages kept at $24 \pm 1\,^{\circ}$C under a 12 h/12 h light–dark cycle and allowed free access to normal food and water. Then, the rats were divided into four groups: the control group (GP, normal diet), SI group (SI, normal diet), ZD group (GP, zinc-deficient diet), and SI + ZD group (SI, zinc-deficient diet). After 1 week (at the age of 4 weeks), ZD was induced in the relevant rats by switching their food to a zinc-deficient diet. The zinc-deficient diet was purchased from Oriental Yeast Co. Ltd. (Yokohama, Japan). The rats in the non-ZD groups were fed the normal diet for a further 2 weeks (to the age of 6 weeks). At the age of 6 weeks, all of the rats were subjected to the elevated plus maze test followed by the forced swim test. After these behavioral tests, the rats' body weight was measured, and blood samples were collected. The rats' blood zinc concentrations were measured using an ESPA·Zn kit (Nipro Corp., Osaka, Japan), and their blood MHPG concentrations were measured as described previously using high-pressure liquid chromatography (HPLC) and an electrochemical detection (ECD) method [23]. Brain NE concentrations and NET expression levels were evaluated in 6-week-old rats (different rats from those used for the behavioral tests).

2.2. Elevated plus maze test

To evaluate anxiety-related behavior, the elevated plus maze test was performed as described previously [24]. Briefly, the device had two open arms (45 cm × 10 cm) and two enclosed arms (45 cm × 10 cm, surrounded by 50 cm high non-transparent walls) extending from a central platform (10 cm × 10 cm), which was elevated 65 cm above the floor. Individual rats were placed on the central platform facing an enclosed arm and were allowed to explore the maze freely for 5 min. Each rat's behavior was monitored using a video camera and was analyzed using the ANY-maze video-tracking system (Stoelting Co., Wood Dale, IL, USA). The total time spent in each area, and the total distance traveled was measured. The ratio (%) of the time spent in the open arms to the total time spent in all arms (%open) was calculated as an anxiety index.

2.3. Forced swim test

To evaluate depressive-like behavior, the forced swim test was performed as described previously [25]. Briefly, each rat was subjected to two sessions. During the first (habituation) session, each rat was placed into a plastic cylinder (height: 80 cm, diameter: 45 cm) maintained at $25 \pm 1\,^{\circ}$C containing 25 cm water for 15 min on the day after the elevated plus maze test. Twenty-four hours later, each rat was placed into the cylinder for a further 5 min, and their behavior was digitally recorded so that it could be scored later. Behavior was analyzed in 5-second intervals, and immobility time, which was defined as the total duration of periods of inactivity, except for movements made by the rats to keep their heads above water, was scored as an index of depressive-like behavior. Three people independently measured immobility time, and the mean time was used.

2.4. Brain NE concentrations

Brain NE concentrations were measured by HPLC combined with ECD (HTEC-500, Eicom, Kyoto, Japan). The rats' brains were excised and divided into six parts: the frontal cortex, striatum, thalamus, hippocampus, cerebellum, and pons. As an internal standard, 100 ng of dihydroxybenzamidine (DHBA) was added to the samples, which were then homogenized in a Polytron homogenizer in 10 volumes of 0.1 N $HClO_4$, and the resultant homogenates were centrifuged at $20{,}000 \times g$ for 15 min. The NE concentration of the supernatant was measured by HPLC with ECD. NE concentrations were calculated using a standard curve constructed with known amounts of NE.

2.5. NET autoradiography

The synthesis of a radioligand for NET ((S,S)-2-(α-(2-[^{77}Br]bromophenoxy)benzyl)morpholine, (S,S)-[^{77}Br]BPBM) and the evaluation of NET expression using autoradiography were performed as described previously [26,27]. Briefly, Br-77 was produced using a low-energy proton reaction involving ^{77}Se(p, n)^{77}Br. The synthesis of no-carrier-added (S,S)-[^{77}Br]BPBM was then carried out via an iodine-radio-bromine exchange reaction. The radiochemical yield of (S,S)-[^{77}Br]BPBM was about 45%, and