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Taurine and β-alanine intraperitoneal injection in lactating mice modifies the growth and behavior of offspring

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ABSTRACT

Taurine, one of the sulfur-containing amino acids, has several functions *in vivo*. It has been reported that taurine acts on γ-aminobutyric acid receptors as an agonist and to promote inhibitory neurotransmission. Milk, especially colostrum, contains taurine and it is known that milk taurine is essential for the normal development of offspring. β-Alanine is transported via a taurine transporter and a protein-assisted amino acid transporter, the same ones that transport taurine. The present study aimed to investigate whether the growth and behavior of offspring could be altered by modification of the taurine concentration in milk. Pregnant ICR mice were separated into 3 groups: 1) a control group, 2) a taurine group, and 3) a β-alanine group. During the lactation periods, dams were administered, respectively, with 0.9% saline (10 ml/kg, i.p.), taurine dissolved in 0.9% saline (43 mg/10 ml/kg, i.p.), or β-alanine dissolved in 0.9% saline (31 mg/10 ml/kg, i.p.). Interestingly, the taurine concentration in milk was significantly decreased by the administration of β-alanine, but not altered by the taurine treatment. The body weight of offspring was significantly lower in the β-alanine group. β-Alanine treatment caused a significant decline in taurine concentration in the brains of offspring, and it was negatively correlated with total distance traveled in the open field test at postnatal day 15. Thus, decreased taurine concentration in the brain induced hyperactivity in offspring. These results suggested that milk taurine may have important role of regulating the growth and behavior of offspring.

1. Introduction

Taurine, one of the sulfur-containing amino acids, is found in the central nervous system of mammals [1] and is essential for the growth and development of neurons [2]. It is well known that taurine has many functions *in vivo*. In addition to those functions, taurine acts on γ-aminobutyric acid (GABA) receptors as an agonist [3] and activates chloride influx into postsynaptic neurons through these receptors [4] in the central nervous system. Moreover, taurine has been shown to activate the corticostriatal pathway as an endogenous ligand for glycine receptors [5]. Thus, it could be assumed that the alteration of taurine levels in the central nervous system may induce some behavioral changes. In fact, taurine administration resulted in anxiolytic-like effects in mice [6]. It has been reported that ICR mice fed with a taurine-supplemented diet for 4 weeks showed a decrease in immobility time in the forced swimming test (FST), and increased anti-depressant-like effects [7] and that Wistar rats fed a high taurine (45.0 mmol/kg) diet for 4 weeks showed decreased immobility in the FST [8]. Furthermore, it has been reported that acute taurine injection reduced locomotor activity; however, chronic taurine supplementation induced a state of neuronal hyper-excitability characterized by increased ambulatory levels and heightened anxiety [9].

Taurine, the end product in the metabolic pathway of sulfur amino acids, is synthesized from methionine and serine or cysteine via cysteic acid and hypotaurine. This synthesis process occurs in the liver, brain, lung and muscle [10–12], but mainly in the liver [13]. However, cysteine dioxygenase (CDO) and cysteinesulfinic acid decarboxylase (CSAD) are expressed in lactating mammary glands [14]. Taurine is found in a high quantity in milk, especially in the colostrum [15], and is essential for the development of

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offspring. This large amount of taurine in the milk seems to derive from: 1) maternal blood via taurine transporters such as the taurine transporter (TauT) and the protein-assisted amino acid transporter (PAT1); and 2) synthesized taurine in the mammary glands [16].

Like taurine, β-alanine is classified into β-amino acids, and they are also very similar in chemical structure. It is well known that β-alanine is transported into each tissue via TauT and PAT1, the same transporters that carry taurine [17].

Therefore, there is a possibility that changes in taurine concentration in milk due to the promotion or suppression of taurine transportation from maternal blood and biosynthesis in the mammary glands may alter the growth and behavior of offspring. The effects of the supplementation of taurine and β-alanine on the growth and behavior of adult animals have been examined. However, few papers have demonstrated the effects of an alteration in the amount of taurine supplied via milk on the growth and behavior of offspring even though milk is the only nutritional source for offspring and clearly important for their development. Thus, the present study aimed to investigate whether the injection of taurine and β-alanine during lactation periods alters the taurine concentrations in milk and whether alterations in taurine concentration affect the growth and behavior of offspring.

2. Materials and methods

2.1. Animals

Pregnant ICR mice (at pregnancy day 13) were purchased from Japan SLC (Hamamatsu, Japan). They were housed individually in a cage (12 cm × 30 cm × 14 cm) and were kept at a constant room temperature of 23 ± 1 °C with humidity at 60% under a 12-h light/dark cycle (lights on at 08:00, lights off at 20:00). At postnatal day 2 (P2), the sex of the offspring was checked, and the number of offspring (n = 8/dam) was standardized in order to make the nutritional conditions the same among the dams. Male offspring were used for body weight measurement and behavioral analysis. The dams were separated into three groups: 1) control group (n = 8); 2) taurine group (n = 8); and 3) β-alanine group (n = 8). Water and a standard diet for laboratory rodents (MF, Oriental Yeast, Tokyo, Japan) were available *ad libitum* for dams and offspring throughout this experiment, which was performed according to the Guidelines for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University, and which conformed to Law No. 105 and Notification No. 6 of the Japanese government.

2.2. Experimental procedures

From P2 to P20, dams in 1) the control group, 2) the taurine group and 3) the β-alanine group were administrated respectively with an intraperitoneal (i.p.) injection of 0.9% saline (10 ml/kg, i.p.), taurine dissolved in 0.9% saline (43 mg/10 ml/kg, i.p.), or β-alanine dissolved in 0.9% saline (31 mg/10 ml/kg, i.p.). The taurine dose was set to 43 mg/kg or 0.34 mmol/kg based on a previous report on taurine. It has been reported that a 43 mg/kg taurine injection reduced locomotor activity [9]. The dose of β-alanine was set to 31 mg/kg or 0.34 mmol/kg, based on the taurine dose. The body weights of 3 male offspring from each dam were measured every 3 days from P5 to P47. Milk samples were collected from dams 90 min after drug administration at P12, and maternal blood samples were collected 65 min after drug administration at P20. Milk samples were stored at −80 °C until analysis took place. Blood samples were centrifuged at 3000 × *g* for 15 min at 4 °C (KUBOTA 3740), and stored at −80 °C until analysis took place. We conducted the open field test on P15 and P36. On P15 and P40, offspring were sacrificed under anesthesia with isoflurane (Escain®, Mylan, Osaka, Japan), and the brain samples were immediately collected. The brain samples were frozen in liquid nitrogen, and stored at −80 °C until analysis took place.

2.3. Milking

Milk samples were collected from the maternal mice from each group on P12, and were used in the analysis of the free amino acid level. We tried to collect milk samples at P1 because colostrum contains a lot of taurine, but we were not able to collect enough milk at this early stage of lactation for amino acid analysis to be carried out. Thus, we chose to collect milk samples later, at P12. Maternal mice and their offspring were separated 6 h before milking in order to collect enough milk. After 6 h, maternal mice were anesthetized with isoflurane and were injected subcutaneously with 0.1 ml of oxytocin to promote the secretion of milk. Ten min after the injection, they were milked for 10–15 min using a KN-591 milking machine for mice and rats (Natsume Seisakusho Co. Ltd, Tokyo, Japan).

2.4. Open field test (OFT)

The taurine concentration in the milk decreased as a result of the β-alanine injection in lactating mice but was not altered by the taurine injection. The motor activity and anxiety-like behavior of male offspring in the control group (n = 8) and the β-alanine group (n = 8) were therefore evaluated on P15 and P36 using the OFT. The test was performed using apparatus, one form of which, used on P15, consisted of a black circular base (diameter 30 cm) with walls that were 10 cm high, while the other, used on P36, consisted of a black circular base (diameter 60 cm) with walls that were 35 cm high. Offspring were tested during the light period and were kept in a closed room at a constant temperature (23 ± 1 °C). The test was performed under light conditions of 100 lux. Each test was recorded on a video recording system for analysis. It was found to be impossible to record the test clearly because of the small body size of offspring at P15, hence the use of different apparatus on P36. At the beginning of the test, a mouse was placed in the center of the apparatus and then allowed to move freely for 5 min. After each trial, the arena was cleaned with 10% ethanol solution to standardize the conditions of all the tests. Open field behavior was analyzed with ANY-maze software (Stoelting Co, Wood Dale, IL) by dividing the field into two zones: an inner zone and an outer zone. Total distance traveled and amount of time spent in the inner zone were measured. The distance traveled was considered to be the parameter for motor activity, and the time spent in the inner zone was considered to be the parameter for anxiety-like behavior.

2.5. Analysis of free amino acids

Free amino acids were analyzed according to the protocol previously reported [7].

2.6. Statistical analysis

All data were expressed as means ± SEM. The body weight of offspring was analyzed by two-way repeated ANOVA. The results of open field tests and the concentration of free amino acids in the brain of offspring were analyzed by two-way ANOVA. All the other results were analyzed by one-way ANOVA. When significant ($P < .05$) effects were detected, comparisons between means were carried out using the Tukey-Kramer test. Differences were considered significant at $P < .05$. All analyses were performed with Stat View (version 5, SAS Institute, Cary, United States, SAS 1998).

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