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Intracerebroventricular Injection of Glutathione Suppresses Food Intake of Neonatal Chicks

[1]H. Yamane, [1]S. Tomonaga, [1]R. Suenaga, [2]D.M. Denbow and [1]M. Furuse
[1]Laboratory of Advanced Animal and Marine Bioresources, Graduate School of Bioresources and Bioenvironmental Sciences, University of Kyushu, Fukuoka 812-8581, Japan
[2]Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0306, USA

Abstract: It is well known that a single deficiency or excess of a Sulfur-containing Amino Acid (SAA), a major determinant of the glutathione concentration in some tissues, decreased food intake of chicks. To clarify the central function of glutathione on food intake, the effect of intracerebroventricular (i.c.v.) injection of reduced Glutathione (GSH) (0.5, 1.0, 2.0 μmol) was investigated in fasting neonatal chicks. Food intake was suppressed by GSH in a dose-dependent manner over 120 min. It was suggested that the reduction in food intake induced by SAA excess, but not SAA deficiency, may be associated with GSH in the brain.

Key words: Reduced glutathione, food intake, intracerebroventricular injection, neonatal chick

INTRODUCTION

Food intake is reduced by an excess or deficiency of a Sulfur-containing Amino Acids (SAA) in growing chicks (Sugahara and Kubo, 1992; Ueda and Tasaki, 1977). Dietary SAA content is a major determinant of glutathione concentration in some tissues since reduced Glutathione (GSH) is a tripeptide which consists of L-glutamate, L-cysteine and glycine. Paterson *et al.* (2001) reported that an SAA deficiency was confirmed by a reduction in liver cysteine and GSH concentrations, decreased food intake and weight loss. Furthermore, the GSH concentration was significantly depressed in the neocortex and thalamus of deficient rats.

GSH acts as an antioxidant and performs detoxication of xenobiotic (Dringen, 2000). In addition, GSH also acts as a neurotransmitter and neuromodulator (Janaky *et al.*, 1999). Hovatta *et al.* (2005) reported that glyoxalase 1 and glutathione reductase 1 regulates anxiety in mice. Glyoxalase 1 is related to detoxication and uses GSH as a cofactor (Thornalley, 2006). Glutathione reductase is related to the antioxidation reaction due to glutathione and catalyses the conversion from oxidized Glutathione (GSSG) to GSH (Dringen, 2000). Therefore, since both enzymes are related to the metabolism of GSH, there may be the relationship between brain GSH and stress responses such as anxiety and fear.

Corticotropin-Releasing Hormone (CRH) is a principal regulator of the hypothalamic-pituitary-adrenal axis (Dunn and Berridge, 1990). In addition to stress-related behaviors, CRH is thought to have as important role in the control of food intake in rats, mice and chicks (Benoit *et al.*, 2000; Contarino *et al.*, 2000; Zhang *et al.*, 2001).

Recently, Yamane *et al.* (2007) found that intracerebroventricular (i.c.v.) injection of GSH dose-dependently induced sleep-like behavior in chicks under acute stressful conditions. Similarly, central administration of Glucagon-Like Peptide-1 (GLP-1) stimulated sleep in neonatal chicks. Food intake was strongly inhibited by GLP-1 (Bungo and Furuse, 2001), an effect that may be due to induction of sleep.

Taken together, GSH may be related to the regulation of food intake. To investigate this possibility, we injected GSH i.c.v. to chicks following a 3 h fasting and determined cumulative food intake for 2 h postinjection.

MATERIALS AND METHODS

One-day-old male layer type chicks (Julia) were purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan) and housed in a windowless room at a constant temperature of 30±1°C. Continuous lighting was provided. The chicks were given free access to a commercial starter diet (AX, Toyohashi Feed and Mills Co Ltd., Aichi, Japan) and water. Chicks were reared in a group until the day before each experiment. On the day of the experiment, chicks (6-day-old) were assigned to treatment groups based on their body weight in order to

Corresponding Author: M. Furuse, Laboratory of Advanced Animal and Marine Bioresources, Graduate School of Bioresource and Bioenvironmental Sciences, University of Kyushu, Fukuoka 812-8581, Japan

produce uniform treatment groups. Experimental procedures followed the guidance for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

GSH was purchased from Sigma (St. Louis, MO). Drugs were dissolved in 0.85% saline containing 0.1% Evans Blue solution and stirred well using a vortex. Saline containing Evans Blue solution was used as a control in all experiments.

The day before an experiment, chicks were individually housed in a cage (185 × 165 × 145 mm, with every 5 cages connected as one unit) in a windowless room. Continuous lighting was provided. The chicks were given free access to feed and water. They were injected i.c.v. with 0.5, 1.0, or 2.0 μmoL GSH or saline after being deprived of food for 3 h. The injected volume was 10 μL. I.c.v. injection was made using a microsyringe according to the method of Davis *et al.* (1979). Cumulative food intake was then measured at 30, 60 and 120 min postinjection.

Data of cumulative food intake at each time period was statistically analyzed by 2 way Analysis of Variance (ANOVA). Significant difference denoted $p<0.05$. Values are presented as means ±S.E.M. Statistical analysis was made using a commercially available package, Stat View (Version 5, SAS Institute, Cary, U.S.A., 1998).

RESULTS AND DISCUSSION

Figure 1 shows cumulative food intake of chicks injected i.c.v. with several doses of GSH after being deprived of food for 3 h. GSH dose-dependently suppressed ($F(3, 27) = 4.867$, $p < 0.01$) food intake at all time periods after the i.c.v. injection of 1.0 and 2.0 μmoL GSH (Fig. 1). Significant regression equations were obtained: Food intake at 30 min (g) = 1.001 (SE 0.178)-0.349 (SE 0.148) GSH ($R^2 = 0.161$, $p<0.05$); at 60 min (g) = 2.000 (SE 0.167)-0.759 (SE 0.139) GSH ($R^2 = 0.508$, $p<0.0001$) and at 120 min (g) = 2.888 (SE 0.241) -0.521 (SE 0.199) GSH ($R^2 = 0.191$, $p<0.05$).

The present study clearly demonstrated that central GSH suppressed food intake in chicks. We also performed a similar experiment with lower doses of GSH (0, 0.025, 0.05, 0.2 μmoL), but no significant effect was found (data not shown). Oxidized glutathione (GSSG) is identified as a sleep promoting substance (Komoda *et al.*, 1990). We confirmed that i.c.v. injection of GSH, as well as GSSG (Yamane *et al.*, 2007), induced sleep-like behavior in neonatal chicks. The percentage of time that chicks exhibited sleep-like behavior during a 10-min behavioral observation was 46.2, 61.3, 76.7 following 0.5, 1.0 and 2.0 μmoL of GSH, respectively (Yamane *et al.*, 2007). It was suggested that GSH or GSSG, which was generated from injected GSH, induced sleep-like behavior. The i.c.v. injection of cysteine and glycine, which are the constituent amino acids of GSH, induced a sedative effect in chicks exposed to isolation stress (Asechi *et al.*, 2006). In addition, L-pipecolic acid, a major metabolic intermediate of L-lysine in the brain, functions not only in the induction of sleep, but also inhibition of food intake in previously fasted chicks (Takagi *et al.*, 2001). Therefore, it is believed that the inhibition of food intake observed in the present experiment was due to the induction of sleep.

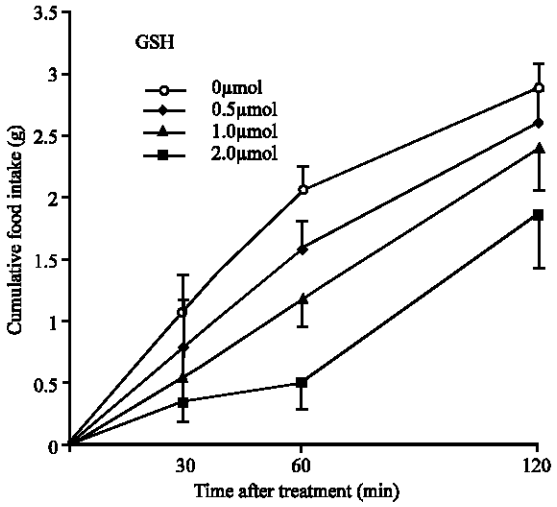

Fig. 1: Cumulative food intake of chicks intracerebroventricularly injected with GSH (0, 0.5, 1, 2 μmol) after a 3 h fast. Results are expressed as means ± S.E.M. The number of chicks used in each group was as follows: 0 μmol, 9; 0.5 μmol, 5; 1 μmol, 8; 2 μmol, 9. Means with different letters at each time are significantly different at $p < 0.05$

CONCLUSION

In conclusion, GSH has an important role for the regulation of feeding behaviors in neonatal chicks. Particularly, GSH may contribute to the reduction of food intake in chicks given a diet high in SAA.

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