

# CHICKEN BREAST EXTRACT AND CARNOSINE IMPROVE LEARNING PERFORMANCE IN MICE

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## Introduction

Carnosine (b-alanyl-L-histidine) and its derivative anserine (b-alanyl-1-methyl-L-histidine) are present at high levels in the muscle and brain of mammalian and avian species, while they are almost negligible in plants (Biffo et al., 1990; O'Dowd et al., 1988; Tamaki et al., 1976). These dipeptides are especially rich in breast muscle of poultry (Nishimura et al., 1988; Aristoy and Toldra, 2004). They have antioxidant activities (Kohen et al., 1988) and buffering capacities (Abe, 2000). In addition, it has been suggested that both dipeptides are putative neurotransmitters in the brain (Tomonaga et al., 2004, 2005).

CBEX (chicken breast extract) is a commercially available supplement rich in carnosine and anserine. Long-term supplementation with CBEX improved high intensity exercise performance (Sato et al., 2003) and relatively high intensity endurance performance (Maemura et al., 2006) in humans. However, the effects of CBEX on the brain function have not been fully investigated yet.

In the present study, whether supplementation of CBEX or carnosine affects learning performance was investigated in mice.

## Materials and Methods

Male mice (six-week-old, ICR strain) were purchased from Japan SLC, Inc. They were kept at 24°C on a 12L:12D light dark cycle (lights on 08:00 h, lights off 20:00 h), and given free access to a commercial diet (MF, Oriental Yeast, Co., Ltd., Tokyo, Japan) and water. They were allowed to habituate for one week before beginning the experiment. This study was performed according to the guidance for Animal Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government. CBEX was produced by Nippon Meat Packers (Tsukuba, Japan). L-Carnosine was purchased from Sigma (St. Louis, USA).

Following a habituation period, mice were randomly selected and divided into two groups, seven to nine mice each. Mice were orally administered with CBEX (5 ml/kg/day) in Experiment 1 or carnosine (0.8 mmol/5 ml/kg/day) in Experiment 2 for five days. Control mice were orally administered with distilled water (5 ml/kg/day). Open field test was done in the last two days 90 min post administration. The open field consisted of a circular area (60 cm diameter and 35 cm high) made from black takiflex. The procedure followed the previous paper (Thiel et al., 1999) with some modifications. Mice were placed at the center of the area. To enable memory consolidation, they were allowed to explore freely the new environment for 5 min under 100 lx lighting. Thereafter, the mouse was returned to the home cage. The open field was cleaned with 70 % ethanol after each test. This experiment was performed twice at 24 h intervals. The distance of path was analyzed automatically with a computer-based video tracking system (AXIS-90, Neuroscience, Inc., Tokyo, Japan). Learning performances were investigated in the percentage reduction of path distance between the first and second trials.

T-test was used for comparisons between means in the percentage reduction of path distance. Statistical analysis was conducted using a commercially available package StatView (SAS 1998).

## Results

Fig. 1 shows effects of CBEX administration on the percentage reduction of path distance in mice in Experiment 1. CBEX tended to increase ( $P=0.078$ ) percentage reduction of path distance compared to the control.

Fig. 2 shows effects of carnosine treatment on learning performances in mice in Experiment 2. Carnosine significantly ( $P<0.05$ ) increased percentage reduction of path distance compared to the control.

## Discussion

The present results demonstrated that orally administered CBEX tended to improve learning behavior in mice. To clarify the factor in CBEX, we focused on carnosine, since carnosine is one of the major constituents

of CBEX in Experiment 2. Carnosine clearly improved learning behavior in mice. Therefore, the function of CBEX on learning behavior may be, at least in part, due to its constituent carnosine. In the previous study, carnosine stimulated nitric oxide generation in the brain (Tomonaga et al., 2005) and nitric oxide in the brain could induce improvement of learning and memory (Thomas, 2000). Thus, the results obtained here may be associated with nitric oxide generation by carnosine while how carnosine in the brain stimulates nitric oxide generation has not been clarified yet. Further studies should be done to clarify the mechanisms by which CBEX and carnosine function on the learning behavior.

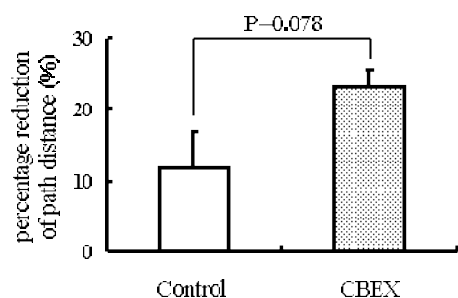


Fig. 1. Effect of CBEX on the percentage reduction of path distance in mice. The numbers of mice used were: control, eight, CBEX, seven. Data are expressed as means  $\pm$  standard error of the mean.

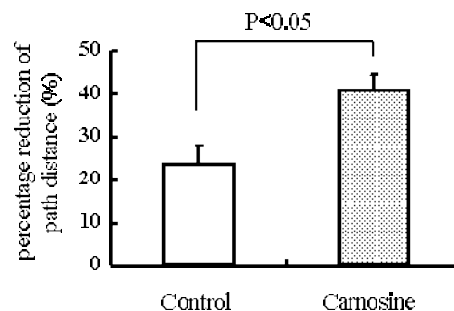


Fig. 2. Effect of carnosine on the percentage reduction of path distance in mice. The numbers of mice used were: control, nine; carnosine, eight. Data are expressed as means  $\pm$  standard error of the mean.

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