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Oral Administration of Chicken Breast Extract Activates Serotonin Metabolism in the Hippocampus of Rats

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Abstract: We previously reported that Chicken Breast Extract (CBEX) had an antidepressant-like effect in rats. In the present study to further clarify, the mechanism of action of CBEX, the effects of CBEX on the brain serotonin (5-HT) system was investigated in the ventral hippocampus of rats, using an *in vivo* micro dialysis. 5-Hydroxyindoleacetic acid, a major metabolite of serotonin, was significantly increased in animals orally administered CBEX. This result indicates that CBEX may activate 5-HT metabolism in the brain. Since, a previous study indicated that orally administered CBEX increases levels of the putative neurotransmitter carnosine in the hippocampus, carnosine might be involved in the action of CBEX.

Key words: Chicken breast extract, hippocampus, serotonin, 5-hydroxyindoleacetic acid, rats, micro dialysis

INTRODUCTION

Chicken Breast Extract (CBEX) is a commercially available supplement rich in carnosine and anserine (Tomonaga *et al*., 2007). Long-term supplementation with CBEX has been shown to improve high-intensity exercise performance (Sato *et al*., 2003) as well as relatively high-intensity endurance performance in humans (Maemura *et al*., 2006). We previously suggested that oral administration of CBEX could increase carnosine and anserine levels and stimulate nitric oxide generation as measured by citrulline production in the hypothalamus and hippocampus of rats (Tomonaga *et al*., 2007). Furthermore, orally administered CBEX had an antidepressant-like effect in rats and there were indications carnosine was involved (Tomonaga *et al*., 2008). However, the mechanisms involved in CBEX action in the brain have not been adequately clarified.

In the present study, to further clarify the mechanism of antidepressant-like action of CBEX, we examined whether orally administered CBEX influences the serotonin (5-HT) system in the brain of rats, as activation of the 5-HT system in the brain is important in the treatment of depression (Ansorge *et al*., 2007). We used an *in vivo* brain micro dialysis method and measured levels of 5-Hydroxyindoleacetic Acid (5-HIAA), a major metabolite of 5-HT, which is representative of 5-HT turnover (Hayashi *et al*., 2003). In the brain, we focused on the hippocampus because this region is important in the pathophysiology of depression (Campbell and MacQueen, 2004).

MATERIALS AND METHODS

Male Wistar rats (6 weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were kept in cages (4 cage^{-1}) in a room at 25±1°C on a 12:12 light-dark cycle (lights on at 8:00, lights off at 20:00) and given free access to a commercial diet (MF; Oriental Yeast, Tokyo, Japan) and water. They were allowed to habituate for 1 week prior to beginning the experiments. This study was performed according to the National Research Council publication, Guide for Care and Use of Laboratory Animals and guidance for Animal Experiments for the Faculty of Agriculture and for the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

CBEX was a gift from Nippon Meat Packers (Tsukuba, Japan). Sodium acetate trihydrate was purchased from Wako (Osaka, Japan). Sodium-1-octane sulfonate was purchased from Nacalai Tesque (Kyoto, Japan). Disodium ethylenediaminetetraacetic acid

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was purchased from Dojindo (Kumamoto, Japan). All other drugs, for which no manufacturer is noted, were purchased from Sigma (St. Louis, MO, USA).

The rats were anesthetized with sodium pentobarbital (45 mg kg^{-1}) intraperitoneally (Dainippon Sumitomo Pharma, Osaka, Japan) and placed in a stereotaxic frame (KN-398-2, Natsume Seisakusho, Tokyo, Japan). Guide cannulas (AG-8, Eicom, Kyoto, Japan) were implanted into the rats ventral hippocampus according to Paxinos and Watson (1997). The coordinates were: 5.8 mm posterior to bregma, lateral -4.8 and 6.8 mm ventral to dura. Body temperature was maintained during the surgery using a disposable pocket warmer. The implanted cannula was then secured with a dummy cannula (AD-8, Eicom) kept in place by a cap (AC-1, Eicom) and each rat was returned to the home cage.

After allowing for 3 days of recovery, the micro dialysis experiment was started at 09:00 and the rats were transferred to an acryl cage (30×30×35 cm). One hour after habituation, rats were anaesthetized with isoflurane (Merck Hoei, Osaka, Japan) and a micro dialysis probe (A-I-8-2, Eicom) with a 2 mm long semipermeable membrane tip was inserted into the implanted guide cannula. The implanted probe was perfused for 8 h with Ringer's solution (NaCl 147 mM, KCl 4 mM, 2.3 mM $CaCl_2$) at a flow rate of 2 μL min^{-1}. After 2 h acclimation, the subsequent 2 h were used to establish a baseline. CBEX or distilled water (control) was then administered orally and investigation of the effect occurred during the subsequent 4 h. Dialysate aliquots were collected every 30 min (60 μL) into the fraction collector (Eicom, EFC-82). The tube containing the dialysate sample was moved to the autosampler (model-231XL, GILSON, Middleton, WI, USA) and the dialysate samples (30 μL) were automatically injected into a High Performance Liquid Chromatography (HPLC) system (Eicom) with a 150×2.1 mm Octadecyl Silane (ODS) column (SC-5ODS, Eicom) and Electrochemical Detector (ECD-300, Eicom) at an applied potential of +700 mV versus an Ag/AgCl reference analytical electrode as described previously by Saito *et al.* (2004). The changes in electric current (nA) were recorded in a computer using an interface system (power chrom ver. 2.3.2 J; AD instruments, Tokyo, Japan). The mobile phase was composed of aceto-citric acid buffer (pH 3.5, 0.1 mol L^{-1}), methanol, sodium-1-octane sulfonate (0.46 mol L^{-1}) and disodium ethylenediamine-tetraacetic acid (0.015 mmol L^{-1}) (830:170:1.9:1) at a flow rate of 0.2 mL min^{-1}. Using this assay, the concentrations of 5-HT, 5-HIAA, dopamine, norepinephrine, dihydroxyphenylacetic acid and homovanillic acid could potentially be calculated. However in the present study, only 5-HIAA is above the detection limit and thus, only its levels were calculated. The detection limit of the system for all monoamines was 0.1 pg $sample^{-1}$.

Following the end of the study, rats were euthanized with an overdose of sodium pentobarbital and a probe soaked with black ink was inserted into the implanted guide cannula for 30 sec. The brains of the animals were subsequently removed and fixed in 10% formalin and the implantation sites of the probe were histologically confirmed.

Analysis for the time course effect data was conducted using a two-way analysis of variance. For comparison of the Area Under Curve (AUC), we used the t-test. These analysis were performed with StatView (version 5, SAS Institute, Cary, NC, USA). Values for time course effect were individually normalized to the mean of the four baseline values. Values for AUC of CBEX treated were normalized by the mean value of AUC of control. All values were expressed as mean±SEM.

RESULTS

The basal extracellular 5-HIAA level (30 μL pg^{-1}), calculated by means of all individual baseline values (n = 7), was 405±57.

Figure 1 shows the time course effect of single oral administration of CBEX on extracellular 5-HIAA levels in the ventral hippocampus of rats. The effect of CBEX was significant ($F(1, 5) = 28.817$, $p<0.005$). The effect of time was not significant ($F(11, 55) = 0.63$, $p = 0.796$). Interaction between CBEX and time was significant ($F(11, 55) = 2.18$, $p<0.05$).

Figure 2 indicates the effect of single oral administration of CBEX on extracellular 5-HIAA levels in the ventral hippocampus of rats. CBEX treatment significantly increased 5-HIAA levels ($p<0.005$).

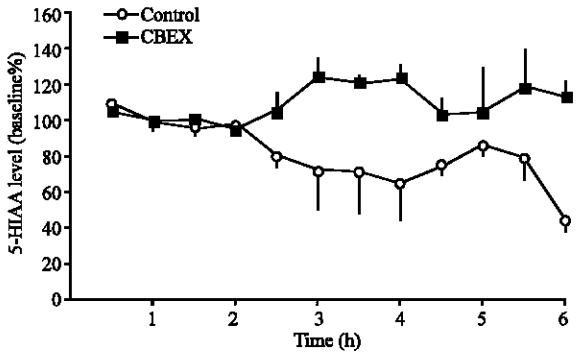

Fig. 1: Time course effect of single oral administration of CBEX on extracellular 5-HIAA in the ventral hippocampus of rats. The number of rats used was: control (distilled water) 4; CBEX (10 mL kg^{-1}) 3. Values are presented as mean±SEM

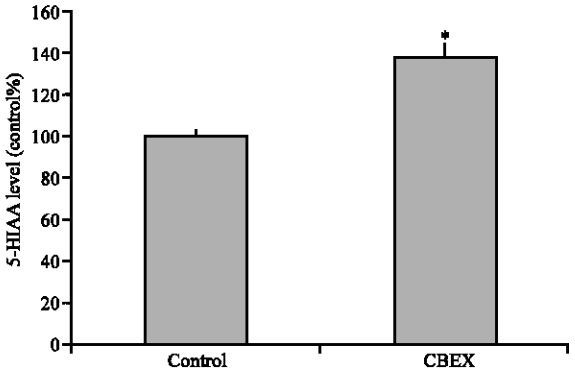

Fig. 2: Effect of single oral administration of CBEX on extracellular 5-HIAA in rat ventral hippocampus. The values were presented by AUC normalized to the mean control value. The number of rats used was: control 4; CBEX (10 mL kg^{-1}) 3. Values are presented as mean±SEM. *Significantly different from the control group ($p<0.005$)

DISCUSSION

In the present study, we confirmed that orally administered CBEX increased the level of 5-HIAA in the ventral hippocampus of rats. 5-HIAA, a major metabolite of 5-HT, reflects central 5-HT turnover (Hayashi *et al*., 2003). Thus, the present result indicates that CBEX stimulates 5-HT metabolism in the ventral hippocampus of rats. In previous studies, an identical dose of CBEX (10 mL kg^{-1}) increased the level of the putative neurotransmitter carnosine (Tomonaga *et al*., 2004, 2005) in the hippocampus of rats (Tomonaga *et al*., 2008). Furthermore, both CBEX and carnosine had an antidepressant-like effect in rats performing the forced swimming test (Tomonaga *et al*., 2008). The antidepressant-like effect seen in the forced swimming test is frequently correlated to activation of the brain 5-HT system (Borsini and Meli, 1988). Therefore, the present result may be connected to carnosine and/or activation of the 5-HT system. To further confirm these results (using an *in vivo* brain microdialysis method), we must employ an assay system that can directly detect 5-HT, thereby demonstrating 5-HT activation. As well, the effect of carnosine administration needs investigation. This will be the basis of the future investigations.

The 5-HIAA levels of the control group decreased after oral administration of distilled water. Under the present experimental conditions, oral administration occurred during the middle of the light phase, while the end of the micro dialysis experiment occurred 2 h prior to the start of the dark phase. This result showed similarities to the previous report, which suggests that Wistar rats, between 1 and 6 months of age, exhibited rhythmic levels of 5-HT in the brain, with maxima during the middle of the light phase and minimum during the middle of the dark phase (Jagota and Kalyani, 2008). However, such a decrease was not observed in CBEX-treated rats; rather, increased levels of 5-HIAA were observed here. Therefore, CBEX may modify circadian rhythms related to the 5-HT system in the brain.

The present result is inconsistent with a previous report indicating that single oral administration of CBEX does not influence 5-HT and 5-HIAA levels in the hippocampus of rats (Tomonaga *et al*., 2008). We speculate that inconsistencies may be due to differences in experimental conditions. In the previous study, the forced swimming test was done twice (pretest and main test) before the hippocampus was collected, while no test was done before and during the microdialysis experiment in the present study.

Alternatively, the possibility remains that in some cases, the levels of 5-HT and/or its metabolite in the whole hippocampus may not reflect neurotransmitter metabolism in the specific region of hippocampus (in this case, the ventral hippocampus). To verify these speculations, further study is required.

CONCLUSION

It is important for the livestock industry to investigate what component in CBEX is important in the present context. As we have indicated, if carnosine is involved in the mechanism, it could enhance the nutritional significance of meat, as carnosine is abundant in meat, while absent in plant material (Aristoy and Toldra, 2004; Tamaki *et al*., 1976; Tomonaga *et al*., 2006). The possibility remains that another component may be involved in the present observations. Further study to evaluate the actions and mechanisms of CBEX on the brain should be undertaken.

Orally administered CBEX can activate serotonin metabolism in the hippocampus of rats.

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