Effects of Chicken Extract on the Serotonergic System of Mice Loaded with Restraint Stress

ABSTRACT
Chicken extract (CE), which is composed of water-soluble substances extracted from chicken by double boiling, could enhance mental efficiency and are helpful to the recovery from postpartum sickness and mental fatigue. But, little information is available regarding its underlying mechanisms. 5-hydroxytryptamine (5-HT), as a central neurotransmitter, involves in various functional changes of brain. In our study, we studied the effects of CE on 5-HT level in restraint-stressed mice. Male Kunming mice were randomly divided into four groups as follows: normal, restraint stress, restraint stress + 12 mL/kg/d CE (CE-L), restraint stress + 24 mL/kg/d CE (CE-H). On the 14th day of administration, all mice were physically restrained in a 50 mL polycarbonate centrifuge tube with holes for 18 h except for normal group. All mice were diethyl ether-anesthetised, 1 day after restraint stress and their brains were obtained for reverse transcription polymerase chain reaction. The levels of 5-HT in plasma, cerebral cortex and hippocampus were also determined by high performance liquid chromatography with an electrochemical detection. The results showed that CE could recover the changed levels of 5-HT in brain or plasma induced by restraint stress, the mechanism may be related to its modulation on tryptophan hydroxylase activity.

KEYWORDS chicken extract; 5-hydroxytryptamine (5-HT); restraint stress

INTRODUCTION
Stress is a state in response to various endogenous and exogenous stimuli. Accumulating evidence indicates that reactive oxygen species generated by occupational and environmental stress exposure are associated with the development of etiology of disease. Recently, with the speeding up of social life rhythm and the changes of living environment, more people have been suffering from stress-caused adverse effects and interest has recently surged in the use of social stress models to illuminate the potential mechanism. For example, stress has been reported to suppress immune system by affecting the secretion of neuroendocrine hormone and causing changes in immunological functions. In addition, several studies in laboratory animals have revealed that restraint stress markedly caused a reduction in spleen lymphocyte number and spleen atrophy, disordered homeostasis of immune function, which increased the susceptibility to pathogens, cancer and neurodegenerative diseases. Furthermore, there have been a number of studies suggesting that stress activates several central neurotransmitter systems, such as histamine, dopamine and serotonin systems, and results in changes of neurotransmitters and their metabolites in brain or plasma. It is now well established that these multiple changes of neurotransmitter systems in stress response are ultimately involved in various functional changes of brain, especially learning and memory function. For instance, stress prior to learning can facilitate or reduce memory. Stress immediately after learning enhances memory; whereas, stress shortly before testing has mainly detrimental effects on memory.

It is well-known that various food affect physiological function. For example, chicken extract, which is composed of water-soluble substances extracted from chicken by double boiling, is a popular health supplement and is consumed particularly by people in China and in Southeast Asia as a traditional health food. Recent studies suggest that it enhances mental efficiency and are helpful to the recovery from postpartum sickness and mental fatigue. It has
been reported that CE contains some beneficial ingredients such as carnosine, anserine, various amino acids, peptides and proteins\textsuperscript{17}. Carnosine, which is a natural antioxidant of meat extract, was suggested to accelerate the metabolism of stress-related substances such as cortisol\textsuperscript{18}.

Kurihara et al. (2006) also indicated the protective effects of CE on energy metabolism disorder in mice loaded with restraint stress through histaminergic neurons\textsuperscript{15}, in which carnosine played a key role. All the above indicate that CE has effects on central nervous system. Thus, we employed mouse physical restraint to study the efficacy of CE on 5-hydroxytryptamine (5-HT) level.

**MATERIALS AND METHODS**

**Chemicals and preparation**

Chicken extract was generously provided by Cerebos Pacific Ltd (Guangzhou, China) as Brand’s Essence of Chicken (70 mL/bottle). It was extracted from chicken meat under high-temperature conditions for several hours, then concentrated and bottled after removing fat\textsuperscript{14}. 5-HT was purchased from Sigma (St. Louis, MO, USA). Gelatin was purchased from Nippo Ltd. (Tokyo, Japan), and was dissolved in water immediately before using.

**Animals**

Male Kunming mice of 7-week-old were purchased from the Center of Laboratory Animal Science Research of Southern Medical University (Guangdong, China). The animals were kept in a specific pathogen-free animal room at 23 ± 1°C and humidity conditions (50–70%) with a 12 h light–dark cycle (lights on from 6:00 to 18:00) under dim white light (about 15 Lux). Animals were allowed to acclimatise to the environment for 1 week before the experiment. Mice were randomly divided into normal, restraint stress, CE-L (restraint stress + 12 mL/kg/d CE) and CE-H (restraint stress + 24 mL/kg/d CE). The CE was concentrated to make sure the intakes of sample were 0.1 mL/10 g body weight. The normal group mice received water only, while restraint stress mice received gelatin, 7.2% gelatin in 0.3% caramel solution with the same caloric as CE. The care and treatment of the animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health (NIH publication no. 85–23, revised 1985), and the experiment was conducted in accordance with animal ethics standards.

**Measurement of 5-HT in the brain tissues and blood**

For plasma analysis, each tube contains 2% sodium heparin. The tubes were centrifuged at 5,000 rpm for 5 min. The supernatant was pipetted off and all samples were stored at −20°C until assay. After drawing blood of mice, their brains were quickly removed and dissected into cortex and hippocampus. These tissues were weighed and added with PBS solution containing 3% perchloric acid whose final concentration was 1 g/mL. Then the tissues were homogenised for 30 s under ice-cold condition, and the homogenate was centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was filtered through a 0.45 µm membrane filter to obtain brain samples. The measurements of 5-HT in blood and brain tissue were determined by HPLC–ECD, as previously reported\textsuperscript{19}.

**Tryptophan hydroxylase (TPH) activity assay**

Supernatant extracts of frozen brain tissues were prepared and the TPH activity was measured, as McCarty reported\textsuperscript{20}. The enzyme reaction was involved conversion of tryptophan to 5-HTP measured in the presence of a decarboxylase inhibitor to prevent further metabolism to 5-HT. The enzyme reaction was terminated with 50 µl of 3% perchloric acid and the precipitated protein was removed by centrifugation, for 30 s at 12,000 g. The 5-HTP production was quantified by electrochemical detection using a HPLC method. Protein was quantified using the Coomassie brilliant blue kit of Nanjing Jiancheng (Nanjing, China). The data are expressed as pmol 5-HTP/mg protein/min.

**Reverse transcription polymerase chain reaction (RT-PCR) analysis of TPH-2 and Serotonin transporter (SERT) mRNA expression**

The mRNA expression of TPH-2 and SERT was determined by RT-PCR method. Total RNAs were extracted from cortex and hippocampus samples using Trizol (Invitrogen, CA). 3 µg of total RNA was reverse-transcribed at 42°C for 1 h in 20 µL reaction mixture containing mouse Moloney leukemia virus reverse transcriptase (Tiangen Biotechnology, Beijing, China) with oligo3 primers (Tiangen Biotechnology, Beijing, China) followed by RT-PCR amplification. Thereafter, cDNA was amplified together with Taq polymerase (Tiangen Biotechnology, Beijing, China) using specific primers with 35 cycles at 94°C for 30 s, annealing temperature at 58°C for 50 s and 72°C for 50 s, followed by incubation at 72°C for 7 min. The RT-PCR primers for mouse TPH-2 mRNA were (F) 5′-TCCAAACTCTACCCTACGCCACTC-3′ and (R) 5′-AACCTGTGTCATACGGC-3′, and the product size was 458 base pairs. Primers for SERT mRNA were (F) 5′-GTTGTCGTGTTGTTGTTG-3′ and (R) 5′-TCCGTTGGTTGTTTCAG-3′, and the product size was 330 base pairs. The primers for the mouse housekeeping gene 18S mRNA were (F) 5′-AGGGGAGAGCCGGTTAGAGGA-3′ and (R) 5′-GGACGGACTAAGCGAGGAACAGG-3′, and the product size was 241 base pairs. The RT-PCR products were fractionated on a
1% agarose gel and visualised by ethidium bromide staining. Band intensity of ethidium bromide fluorescence was measured by using BIO-RAD Image Analysis system (Bio-Rad, Hercules, CA), then quantified by Quantity One analysis software (Bio-Rad, Hercules, CA) and expressed as the ratios to 18S.

**Statistical analysis**

Each datum was represented as mean ± SD. Significant differences between the two groups were analysed with Student’s t-test. One-way analysis of variance (ANOVA) was applied to analyse differences in data of biochemical parameters among the experimental groups, followed by Dunnett’s test for pair-wise multiple comparisons. Differences were considered as statistically significant at \( P < 0.05 \).

**RESULTS**

**Effects of CE on 5-HT levels in brain regions and blood of restraint-stressed mice**

5-HT, as a central neurotransmitter, involves in various functional changes of brain. In this study, 5-HT in the brain tissues and blood were examined. As shown in Fig. 1, the chronological changes of 5-HT levels in brain cortex and hippocampus of restraint-stressed mice were determined by HPLC–ECD. Compared with the normal control mice, the 5-HT levels increased by restraint for 8 or 12 h, but decreased by restraint for 18 or 24 h. As shown in Fig. 2, 5-HT level obviously was decreased in brain regions and plasma by restraint for 18 h, but was increased by the administration of CE (12 and 24 mL/kg/d) significantly.

![Fig. 1](image1.png)

**Fig. 1** Chronological changes of 5-HT levels in mice brain cortex and hippocampus of restraint-stressed mice. Data were expressed as means ± SD (n = 7). The difference was considered statistically significant at \(* P < 0.05, ** P < 0.01\) vs. normal control group.

![Fig. 2](image2.png)

**Fig. 2** Effects of CE on 5-HT levels in brain regions and blood of restraint-stressed mice. Data were expressed as means ± SD (n = 9). The difference was considered statistically significant at \( \triangle \triangle P < 0.01\) vs. normal control group; \(* P < 0.05, ** P < 0.01\) vs. restraint stress group.
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**Effects of CE on TPH activity in brain regions of restraint-stressed mice**

TPH is the limited enzyme in the synthesis process of 5-HT. In order to investigate whether the elevated 5-HT levels in CE-treated mice were the consequence of the enhanced TPH activity, TPH activity assay were conducted. As shown in Fig. 3, there was a significant decrease in the TPH activity as indicated by a decreased rate of synthesis of 5-HTP in cortex and hippocampus from restraint-stressed mice. However, administration of CE (12 and 24 mL/kg/d) significantly increased the TPH activity. These observations strongly suggested that the increased 5-HT level by CE might be related to an enhanced in the TPH activity.

**Effects of CE on mRNA expression of TPH-2 and SERT in brain regions of restraint stressed mice**

There are two distinct TPH genes encode two different homologous enzymes TPH1 and TPH2. TPH2 is exclusively expressed in neuronal cell types and is the predominant isofrom in the central nervous system. The SERT, a monoamine transporter protein, reuptakes serotonin in the synaptic cleft and terminates its function. The mRNA expression of TPH-2 and SERT was determined by RT-PCR method. As shown in Fig. 4, the mRNA expression of TPH-2 (Fig. 4a) was down-regulated both in cortex and hippocampus of mice loaded with restraint stress, but the mRNA expression of SERT (Fig. 4b) was not changed. However, administration of CE (12 and 24 mL/kg/d) significantly up-regulated the mRNA expression of TPH-2 both in cortex and hippocampus of mice loaded with restraint stress. It can be inferred that restraint stress-induced changes in 5-HT level might be associated with down-regulation of TPH-2 mRNA expression independent of SERT, and EC could recover the changed levels of 5-HT by elevating TPH-2 mRNA expression.

**DISCUSSION**

The brain is the master of behavioural and physiological process in stress response. Previous studies suggested the changes of central neurotransmitter systems in brain may account for the abnormal learning and memory function in restraint stressed mice. Moreover, Amat et al. (1998) suggested various stress loadings might affect changes in 5-HT levels. Our previous study also showed Brand’s Essence of Chicken had anti-stress effects on energy metabolic disorder in restrain stressed mice, which was suggested to be related with 5-HT system through its H1, H2 and H3 receptors. Hence, we employed restraint stress to investigate the effects of EC on 5-HT level. The results in this study showed that, when compared with control mice, 5-HT level in the cerebral cortex, hypothalamus and plasma were significantly decreased in restraint stressed mice. The results suggested that restraint stress could stimulate metabolism of central 5-HT. However, the 5-HT levels in the CE group were significantly increased in the cerebral cortex, hypothalamus and plasma. Moreover, Xu showed that the Essence of Chicken extract increased cerebrospinal fluid level of 5-hydroxyindole acetic acid in animals. In addition, the decrease of 5-HT levels may be related to reducing tryptophan availability and TPH activity in brain when animal was exposed to acute stress, which was in accordance with our study.

All the above indicates that CE has recovery effects on 5-HT level through increasing the mRNA expression of
TPH-2 in the central nervous system in restraint stressed mice to modulate TPH activities. The mechanism may be related to its beneficial ingredients, such as carnosine, anserine, various amino acids, peptides and proteins, which has been demonstrated to maintain the homeostasis of internal environment in the body through its anti-stress effects.11,13,22,23

**REFERENCES**


**Fig. 4** Effects of EC on mRNA expression of TPH-2 and SERT in brain regions of restraint stressed mice. (A) and (B) Representative of RT-PCR analysis of TPH-2 and SERT respectively. (C) and (D) Densiometric analysis of PCR products of TPH-2 in mice hippocampus and cortex, respectively. The results were generated as relative intensity units by densitometry and expressed as the ratio of TPH-2 to 18S. The data were expressed as means ± SD (n = 7). The difference was considered statistically significant at ∆∆P < 0.01 vs. normal control group; *P < 0.05, **P < 0.01 vs. restraint stress group.
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