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Effects of different processed meat proteins on the production of serotonin (#610)

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Introduction

The gut–brain axis is a bidirectional communication system between the central nervous system and the gastrointestinal tract. Serotonin can activate intrinsic and extrinsic primary afferent neurons, and further influence intestinal peristalsis, motility, nutrients absorption and information transit to the central nervous system. More than 90% of the body's serotonin is produced in the gut and particularly in enterochromaffin (EC) cells through the tryptophan 1 hydroxylase 1 (Tph1) enzyme. Meat products are a good source for dietary proteins. However, the proteins are prone to chemical changes during meat processing, which may affect the digestion and absorption in the digestive tract. Some studies indicated that EC cells have the ability to respond to the chemical composition of the luminal contents by releasing serotonin. The aim of this work was to explore the effects of different processed meat proteins on the production of serotonin.

Methods

Animals and diets

A total of 60 4-week-old male C57BL/6J mice were raised in a specific pathogen-free animal center. Mice were housed in a controlled environment (60±10% of humidity, 12 h light cycle, 20.0±0.5 °C) with free access to water and diet. Mice were fed a standard chow diet during a 2-week acclimation period. Then, animals were divided into six groups and fed for 8 months with one of six protein diets, that is, casein (C), soy protein (SP), emulsion-type sausage protein (ESP), dry-cured pork protein (DPP), stewed pork protein (SPP) and cooked pork protein (CPP). After eight-month feeding, mice were euthanized by cervical dislocation. The gut tissues were taken and subjected to fixation in 4% (wt/vol) paraformaldehyde or were stored at −80°C.

Chemical analyses

The 5-HT concentration in tissue was quantified using a ELISA kit. Readings from tissue samples were normalized to total protein content that was quantified using a BCA protein assay kit.

Histological observations

After being fixed in 4% paraformaldehyde buffer for 24 h, the tissues were transferred to graded series of ethanol and cleaned by xylene and then embedded in paraffin. The paraffin-embedded tissues were cut into 3 m thick sections then the sections were fixed, dewaxed, hydrated, antigen repaired and closed. Then the sections were incubated with rat anti-serotonin primary antibody. The sections were washed and incubated for 1 h with goat anti-rat Dylight 488-conjugated IgG. The nuclei were labeled with DAPI before sealing with a coverslip. The images were captured using an AxioImager microscope. Finally, immunoreactive cells were enumerated in 10 visual high-power filed from each sample.

Western blotting

The frozen samples were ground in liquid nitrogen and lysed in protein extract buffer. The protein concentration was determined and SDS-PAGE was run. Proteins were transferred to polyvinylidene difluoride membrane and blocked in 5% fat-free milk for 1 h. Samples were incubated individually with antibodies for chromogranin A, serotonin transporter, monoamine oxidase A, glyceraldehyde 3-phosphate dehydrogenase, and tryptophan hydroxylase 1 at 4 °C overnight. Then the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The membranes were washed and reacted with ECL. Images were acquired and band density was quantified. The intensities of target proteins were normalized against that of GAPDH.

RT-qPCR

Total RNA was isolated from tissues and used to prepare cDNA. RT-qPCR was performed by the SYBR Green probe. The mRNA level was normalized relative to GAPDH and calculated as fold ($2^{-\Delta\Delta Ct}$) in which the casein group was set as control.

Results

The serotonin level decreased gradually from the duodenum to the cecum, which was followed by a substantial increase in the colon (Fig.1A). In the duodenum, the CPP and DPP groups had higher serotonin levels than the C group. In the jejunum, the average serotonin level was the lowest for the SPP group. In the cecum, serotonin levels were the lowest for the ESP group but the highest for the C group. However, the ESP group had the highest serotonin value in the colon. Chga was upregulated in the cecum by the C and DPP diets (Fig.1B), but it was upregulated in the colon by the ESP diet (Fig.1C). The differences in serotonin and Chga levels may be related to the number of EC cells. The immunohistological observations indicated that the number of EC cells in the cecum were lower in the ESP group than those of the casein and DPP groups (Fig.2A). The ESP group had a lower number of EC cells in the colon (Fig.2B). These changes may be related to gut microbiota. The relative abundances of Tph1 mRNA and protein were the highest in the SP group but the lowest in the C group (Fig.3A). The DPP diet upregulated SERT and Maoa gene expression compared with the casein group (Fig.3B and C). Different processed meat proteins significantly affect the ex

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pression of catabolism and anabolic related enzymes and transporters.

Conclusion

Different processed meat proteins regulate serotonin levels by affecting the number of serotonin-secreting cells, catabolism, anabolism, and transporters. This may be related to gut microbiota, and the specific mechanism needs further investigation.

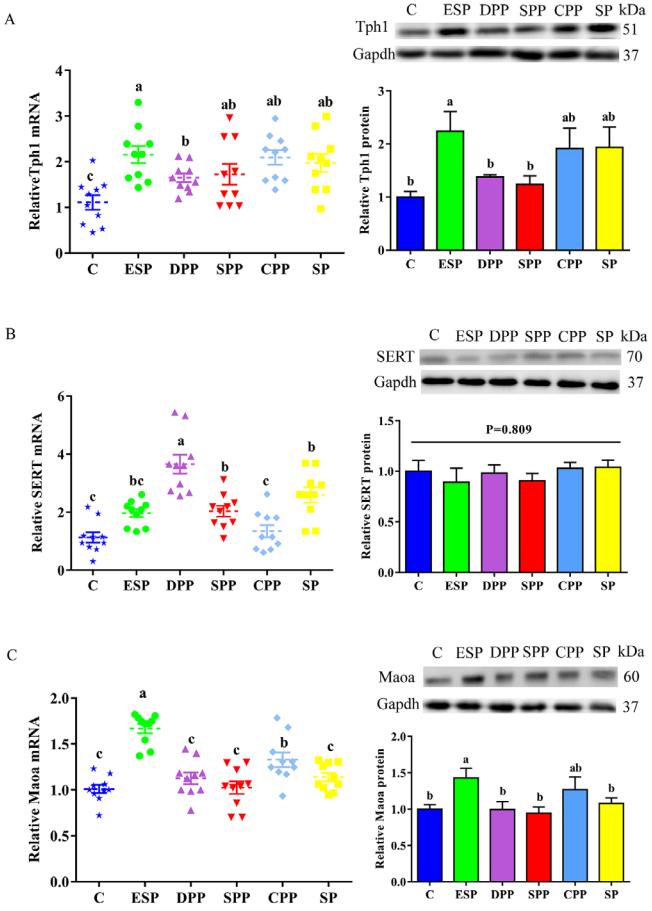

Figure 3. Effects of protein diets on 5-HT synthesis, catabolism or reuptake in colon. (A) Tph1 mRNA and protein expression. (B) SERT mRNA and protein expression. (C) Maoa mRNA and protein expression. Note: the data were analyzed by ANOVA and means were compared by the procedure of Duncan's multiple-range comparison. The "a, b, c" means with different letters differed significantly ($P<0.05$).

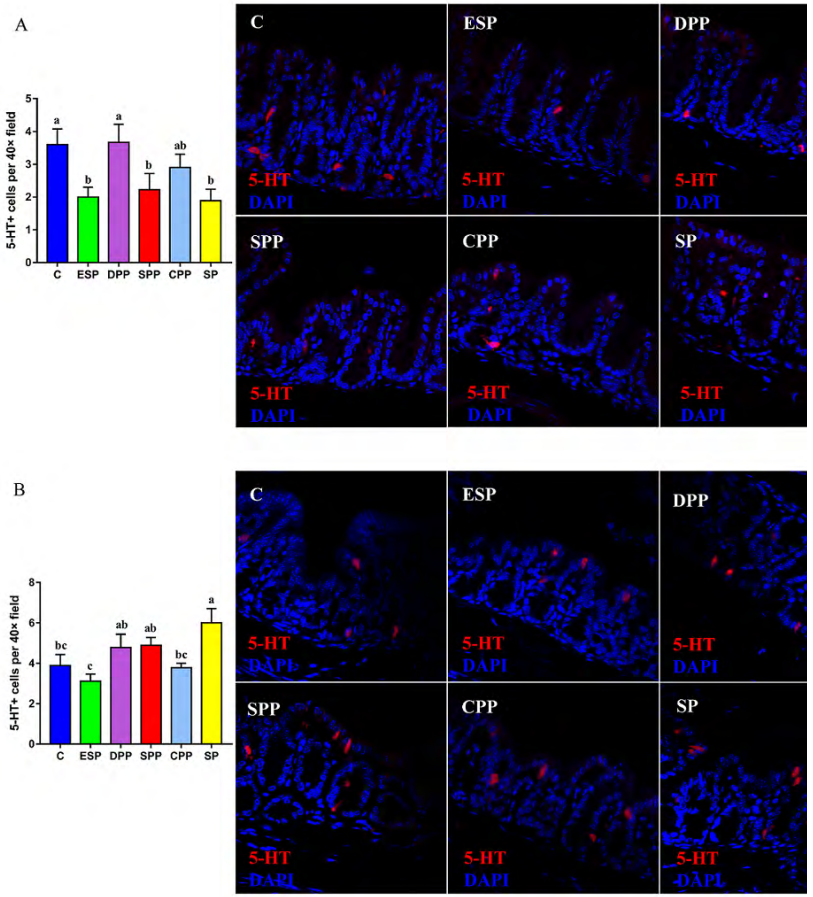

Figure 2. Enterochromaffin cells density in cecum and colon. (A) Cecum. (B) Colon. EC cells were identified by immunofluorescence detection of 5-HT (red, Cy3-labeled 5-HT+ cells; blue, nuclei counterstained with DAPI). The data were analyzed by ANOVA and means were compared by the procedure of Duncan's multiple-range comparison. The "a, b, c" means with different letters differed significantly ($P<0.05$).

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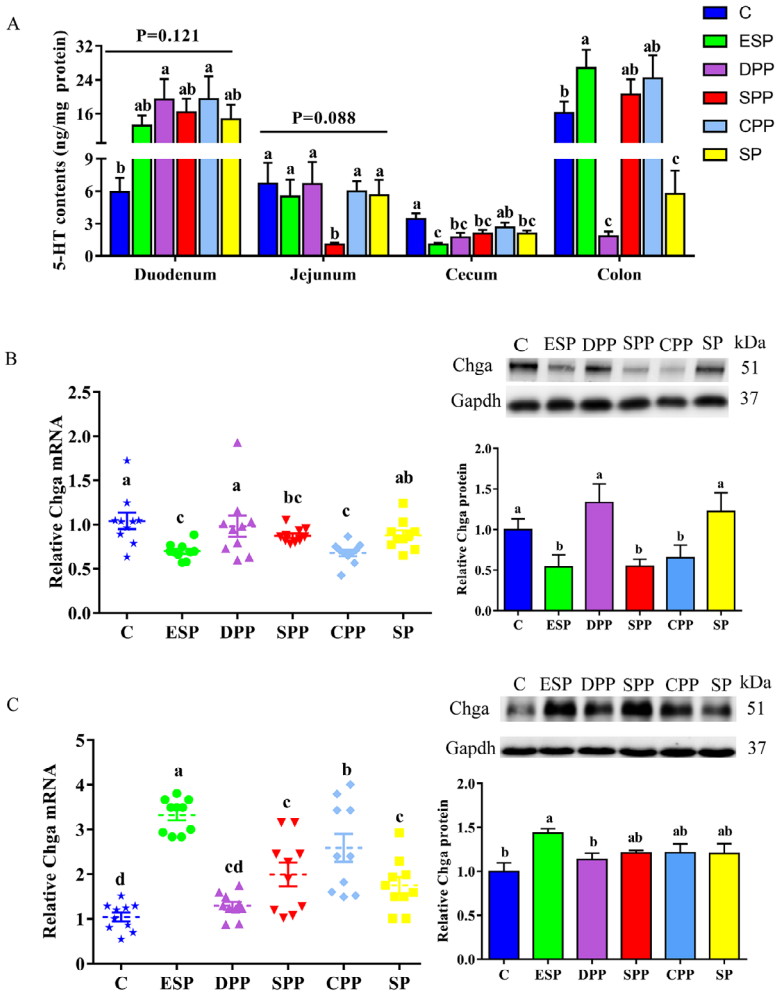

Figure 1. Variations in the 5-HT content and Chga expression in intestinal tissues.
(A) 5-HT content of intestinal tissues. (B) Chga mRNA and protein expression of cecum. (C) Chga mRNA and protein expression of colon. The data were analyzed by ANOVA and means were compared by the procedure of Duncan's multiple-range comparison. The "a, b, c" means with different letters differed significantly ($P<0.05$).

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