Note

Effects of Dietary Administration of Plant-Derived Anthocyanin-Rich Colors to Spontaneously Hypertensive Rats

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Summary Anthocyanins have beneficial effects such as free radical scavenging activity. We investigated the effects of continuous administration of colors from purple corn (PCC), purple sweet potato (PSC) and red radish (RRC) to spontaneously hypertensive rats (SHR). These are rich in anthocyanins. Animals were fed with diets containing PCC, PSC or RRC (1 mass% of diets) for 15 wk. While the body weight and the daily food intake of administered rats were not different from those of the non-administered control rats through the experimental period, the blood pressure and the heart rate of SHR administered each color decreased as compared to the control group from the early stage of administration. These results suggest that plant-derived colors containing anthocyanins have anti-hypertensive effects on hypertensive animals.

Key Words anthocyanin, blood pressure, hypertension, administration with diet

Purple corn (Zea mays Linne), purple sweet potato (Ipomoea batatas Poir.) and red radish (Raphanus sativus Linne) colors (PCC, PSC and RRC, respectively) are natural pigments extracted from each plant with warm water or a slightly acid solution. These colors are used as an anthocyanin color for foods. These are unstable both under neutral conditions and alkaline conditions and become discolored soon after they are placed under such conditions. Colors examined in this study mainly include cyanidin-3-glucoside (PCC) (1), cyanidin-acylglucoside or peonidin-acylglucoside (PSC) (2) and pelargonidin-acylglucoside (RRC) (3). Anthocyanins have attracted attentions of researchers for their various beneficial effects on health. It has been reported that anthocyanins have antioxidant activity (4) and other physiological properties including anti-thrombosis (5) and anti-cancer function (6) and that PCC prevents obesity and hyperglycemia (1). It has also been reported that intragastric administration of PCC lowers the blood pressure of spontaneously hypertensive rats (SHR) (7). Similarly hesperidin, a flavonoid in citrus fruit, which has antioxidant activity, reduced the blood pressure of SHR significantly (8). Since PSC and RRC are also rich in anthocyanins, they are expected to have anti-hypertensive activity. In the present study, we assessed the effect of continuous administration of PCC, PSC and RRC in the free access diets on growing SHR whose age was from 5 to 20 wk. In addition, since inhibitory activity on the angiotensin-converting enzyme (ACE) and content of γ-amino-butyric acid (GABA) of these colors were not determined, we conducted measurements of them as well. As mentioned above, these colors are used for coloring foods. GABA (9) or ACE-inhibiting substance (10) in food was reported to lower the blood pressure.

Materials and Methods

Animals and diets. Male SHR (n=25) aged 3 wk were purchased from Charles River Japan Inc. (Kanagawa, Japan) and housed in an air-conditioned room (25°C) with a 12 h light/dark cycle (light: 8:00–20:00). They were given free access to a stock diet (MF, Oriental Yeast, Chiba, Japan) and water. They were divided into four groups, 2 wk later, and each group was fed for 15 wk on respective diets containing PCC, PSC, RRC or non-containing MF diet as control. PCC, PSC, and RRC were supplied by San-El Gen F.F.I., Inc. (Osaka, Japan) and were estimated to contain 26, 38 and 33% anthocyanins, respectively. These colors were added to the diet at a concentration 10 g/kg diet.

The Animal Experiment Committee of Okayama University Faculty of Agriculture approved this experimental design and the rats were managed in line with the Guidelines for Care and Use of Laboratory Animals.

Measurements. Bodyweight, daily food intake, heart rate, systolic blood pressure and mean blood pressure were measured once a week. The blood pressure and the heart rate of pre-warmed (37.5°C) conscious rats were measured using the indirect tail cuff method (BP-98A, Softron, Tokyo, Japan). The mean of at least three successive measurements was taken as the datum for blood pressure and heart rate of the animal.

GABA in colors dissolved with 10 w/v% sulfo-salicylic acid solution was measured using amino acid analyzer (JLC-500/V, JEOL). ACE inhibition was measured as follows. Mixture of colors dissolved in dimethylsulfoxide (1%) and ACE solution (0.06 units/mL, Sigma) was
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pre-incubated for 10 min at 37°C. The substrate, hippuryl-histidyl-leucine (6.7 mM), was added and incubated for 30 min. The hippuric acid liberated by ACE was determined spectrophotometrically at 228 nm.

Statistics. Data are shown as the mean ± standard error (n = 6 or 7). Two-factor ANOVA was applied for statistical analysis and differences in means were considered significant at p < 0.05.

Results

Body weights of SHR increased with age and there was no significant difference between groups administered anthocyanin-rich colors and control rats throughout the experimental period (Fig. 1). The addition of colors to diet did not cause a change in the daily food intake of SHR (data not shown).

The heart rate slightly decreased until 12 wk of age in all groups and was lower in anthocyanin-administered groups as compared to the control group (Fig. 2). There was no difference among three groups administered PCC, PSC or RRC. The changes in systolic blood pressure are shown in Fig. 3. The systolic blood pressure of control SHR continuously increased from 130.9 mmHg of 5-wk-old animals to 233.1 mmHg of 11-wk-old ones. The blood pressures of anthocyanin-administered groups were significantly lower than that of control. PSC- and RRC-administration to SHR more effectively lowered blood pressure as compared to PCC-administration. The mean blood pressure similarly increased from 96.2 mmHg of 5-wk-old SHR in control group to 183.5 mmHg of 11-wk-old ones. As is the case with the systolic blood pressure, the mean blood pressures of three groups administered anthocyanin colors were significantly lower than that of non-administered control group and those of PSC- and RRC-administered SHR were lower than for the PCC group (data not shown).

GABA was not detected in PCC, PSC or RRC (less than 0.001%). In addition, 1% solutions of these colors have no inhibitory activity on ACE.

Discussion

The effects of anthocyanin-rich plant colors extracted from purple corn, purple sweet potato and red radish, PCC, PSC and RRC, were examined in SHR. Each color was mixed into their diet (1%) and was administered for 15 wk. The administration of these colors did not affect
the body weight or the daily food intake of SHR. Intragastric administration of PCC was reported not to affect the body weight of SHR (7). The administration of hesperidin mixed into the diet did not alter the body weight or daily intake of SHR or normotensive Wistar Kyoto rats (8).

As previously reported with PCC (7), it is shown in the present study that the administration of PCC, PSC and RRC in free access diet decreased the systolic and mean blood pressure in SHR from the early stage of administration. Though the principal coloring ingrediment of the three colors examined in this study differs, the major ingredients, cyanidin-3-glucoside, peonidin-acylg glucoside and pelargonidin-acylg glucoside, have similar antioxidative activity (2, 4, 11). This common antioxidant property of these anthocyanin colors may play a role in the hypotensive effect such as the preservation of vasorelaxant endothelial nitric oxide and/or the prevention of low-density lipoprotein oxidation (12). Mendes et al. (13) reported that anthocyanins have an endothelium-dependent inhibitory effect on arterial contraction. It is also reported that cyanidin-3-glucoside up-regulates endothelial nitric oxide synthase (14). The inhibition of serum lipid oxidation is expected to preserve the intact structure of vessel walls (15).

It should be considered that other plant-derived hypotensive ingredients might be involved. The systolic blood pressure of SHR fed a 0.15% GABA diet has been lowered (9). In the present study, GABA was not detected in the three colors. It is reported that peptides from fermented milk inhibited ACE activity and lowered the blood pressure of hypertensive volunteers (10). Although the inhibitory actions of some flavonoids on ACE were investigated (16, 17), we observed no inhibitory activity in PCC, PSC, or RRC.

In this study, the administration of PSC and RRC showed more effective reduction of blood pressure in SHR than that of PCC, PCC, PSC, and RRC used in this study were estimated to contain 26, 38 and 33% anthocyanins, respectively. The reason why the difference among the efficacy of the colors exists may be caused by the difference of the content of anthocyanins. Comparative investigations on the various types of the purified anthocyanins may be able to confirm the effects of anthocyanin-colors.

The heart rate of SHR was also decreased by the administration of anthocyanin colors used in this study. A similar inhibitory effect of a flavonoid on the heart rate was observed in SHR (8). Although the heart rate is thought to relate closely to the blood pressure, the mechanism(s) of the effect on the heart rate needs to be investigate further.

We demonstrated in the present study that the continuous administration of PCC, PSC and RRC to SHR for 15 wk showed an inhibitory effect on the increase of blood pressure. These effects are suggested to depend on a common property of anthocyanins such as antioxidative activity.

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