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Effects of Dietary Protein Source and Umami on the Palatability to Sodium Chloride in Rats

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Summary

Previously we reported that the preference for NaCl is modified with nutritional factors (i.e., dietary protein level). We selected dietary protein sources as nutritional factors and examined the effects of dietary protein sources and umami on the appetite or preference for sodium chloride. Compared with vegetable protein (i.e., isolated soybean protein), the intake of animal protein (i.e., egg protein and meat protein) caused the preference for an aqueous NaCl solution of a lower concentration and as a result, the total salt intake was reduced. When the protein value of the vegetable protein was improved, however, the salt taste preference became similar to the one observed in rats fed animal protein. The blood free glutamic acid concentration was significantly increased by taking vegetable protein. When allowed to select monosodium glutamate (MSG) solutions together with aqueous NaCl solutions, the salt taste preference of rats fed animal protein was further reduced. However, the rats fed vegetable protein never selected any MSG solutions.

It has already been reported, based on studies with the use of rats, that the preference for sodium chloride (NaCl) is modified with genetic factors. Namely, it has been clarified that spontaneously hypertensive rats (SHR) prefer an aqueous NaCl solution of higher concentration, compared with Sprague-Dawley (SD) rats or Wistar rats (1-4). On the other hand, the preference for NaCl seemingly depends on nutritional factors, in addition to the above-mentioned genetic factors. Regarding salt preference, it is known that the salt taste preference is enhanced by fasting for 24 h, restraint (5) and one' s salt-intake career (6). In a previous experiment (7), we selected a dietary protein level as nutritional factor affecting salt taste preference and performed a salt taste preference test by feeding SHR rats and Wistar-Slc rats with a high-protein diet and a low-protein diet. As a result, we reported that the salt taste preference was enhanced as the diet protein level was lowered and the NaCl intake was increased while the preference for a monosodium glutamate (MSG) solution was weakened simultaneously, regardless of strain (7). We further reported that taste cells withered and became hollow and the turnover of taste bud cells was retarded with a decrease in the diet protein level (7).

In this study, we selected dietary protein sources as a nutritional factor and examined the effects of animal and vegetable proteins on the salt taste preference of SHR and Wistar rats. We emploied purified egg protein and pork protein as animal proteins, while isolated soybean protein and an improved protein prepared by adding methionine (i.e., the first restricting amino acid) as vegetable proteins. Furthermore, changes in the salt taste preference due to the coexistence of MSG solutions were also examined.

Materials and Methods

Animals, Diets and Experimental Design Experiment of NaCl Select ion. 〈Experiment 1〉

Male SHR and Wistar-Slc rats (Japan Slc Inc., Shizuoka, Japan : i.e., original animal of SHR) aged 4 weeks were employed as experimental animals. These rats were previously fed a commercially available solid feed (F2, Funabashi Farm Inc., Chiba, Japan) for 1 week and then subjected to the experiment. Table 1 shows the experimental groups and feed compositions. Four experimental groups differing from each other in protein source, namely, a purified egg protein group (PEP group), an isolated soybean protein group (SPI group), an isolated soybean

TABLE 1. *The Composition of Experimental Groups*

Ingredients (%)	Experimental groups			
	PEP	MP	SPI	Met
Purified egg protein	10	—	—	—
Pork meat protein	—	12.5[a]	—	—
Isolated soybean protein	—	—	10	10
L-Methionine	—	—	—	0.3
Corn starch	74	71.5	74	73.7
Soybean oil[b]	5	5	5	5
Salt mixture[c]	4	4	4	4
Vitamin mixture[d]	2	2	2	2
Cellulose powder	5	5	5	5

[a] Addjusting 10% protein level. [b] Vitamin D (20 IU/g) is added. [c] According to Harper's salt mixture. [d] Oriental Mixture, Oriental Yeast Co. Ltd.

protein+0.3% L-methionine group (Met group) and a pork meat protein group (MP group), wherein swine dorsal musculus longissimus was cut into pieces, defatted with hexane and lyophilized, were used. A protein level of 10% was employed. The feeding room was maintained at a temperature of 25±1°C and a humidity of 50±5% and lighting was controlled so as to give a lighting/shading cycle per 12 hr (switch on at 8 : 00 P.M., switch off at 8 : 00 A.M.). Five rats per group were fed in a large wire cage. Deionized water and aqueous NaCl solutions containing 0.5%, 0.9%, 1.4% and 2.0% NaCl were prepared in graduated water-supply tanks located in parallel, and the rats were allowed free choice of these solutions.

Experiment of NaCl Selection Due to the Coexistence of MSG. ⟨*Experiment 2*⟩

Diet composition and experimental design had the same conditions in Experiment 1. 20 mM, 40 mM and 80 mM MSG solutions were added to aqueous NaCl solution series of Experiment 1. Thus the rats were allowed to select any solution from among eight.

Measurement During Experiment

Body weight, water intake and feed intake were measured once a day (at 4 : 00 P.M.). The location of the water-supply tanks were changed at random in order to prevent the location of the tanks from affecting the selection.

Blood Collection and Physicochemical Analysis

After the completion of a selection test each animal was fasted for 12 hr and killed by withdrawing blood from the abdominal aorta under light ethyl ether anesthesia and dissected. The blood was immediately collected in a test tube. The plasma was prepared by a routine method and the free amino acids composition was analyzed by the *o*-Phthalaldehyde method with HPLC (LC-6A, Shimadzu Co., Kyoto, Japan) (8).

Sodium Contents of Experimental Diets

The sodium contents were measured by atomic absorption spectrometry (AA-670, Shimadzu Co., Kyoto, Japan) following ashing and extracting with hydrochloric acid (9) and then converted into the salt contents.

Statistical Analysis

Data were analyzed by the Student's *t*-test (10).

Results

〈*Experiment 1*〉

Regarding growth conditions, the PEP group showed the greatest weight gain while the SPI group showed the least weight gain in both of SHR and Wistar-Slc rats. Fig. 1 shows the time course of preference for various concentrations of aqueous NaCl solutions in SHR. The PEP and MP groups (i.e., the animal protein groups) preferred the 0.9% aqueous NaCl solution the best, the concentrations of which was almost isotonic with those of their bodily fluids, and scarcely took other solutions. On the other hand, the SPI group (i.e., the vegetable protein group) preferred the 1.4% aqueous NaCl solution during the experimental period. In contrast, the Met group fed with the feed of improved amino acid compositions of SPI selected 0.9% and 0.5% aqueous salt solutions. That is to say, the salt taste preference of this Met group was similar to those of the animal protein groups. Similarly, Fig. 2 shows the time course of preference for various concentrations of aqueous NaCl solution in Wistar-Slc rats. The salt intake of all groups in the Wistar-Slc rats were smaller than those of SHR. Similar to SHR, vegetable protein groups preferred aqueous NaCl solutions of higher concentration compared with animal protein groups. Table 2 shows the sodium contents of each experimental diets and feed intakes during the experimental period. The sodium

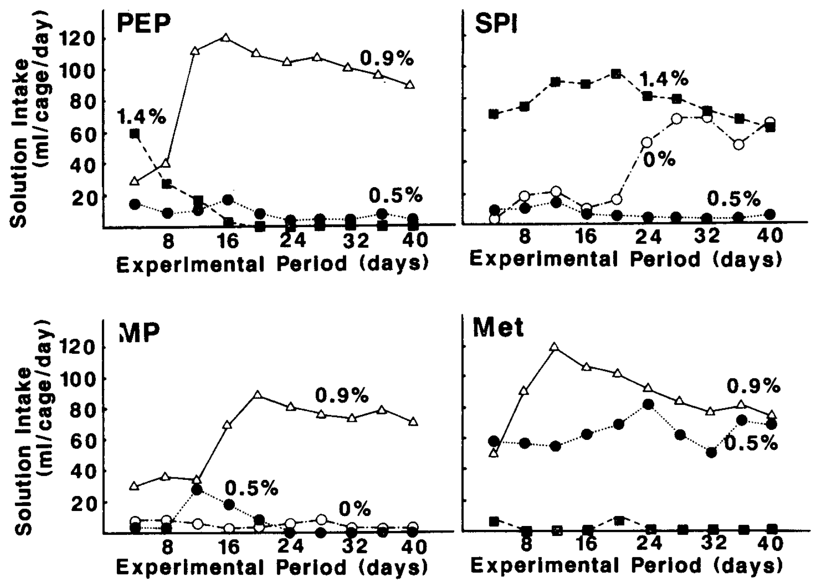

FIG. 1. The time course of preference for various concentrations of aqueous NaCl solutions in SHR fed with four varietes of diet. Solution intakes are expressed as mean values for four days for each group of five rats. Deionized water, ---○---; 0.5% aqueous NaCl solution, ···●···; 0.9% aqueous NaCl solution, —△—; 1.4% aqueous NaCl solution, --■--; 2.0% aqueous NaCl solution was not prefered for every group.

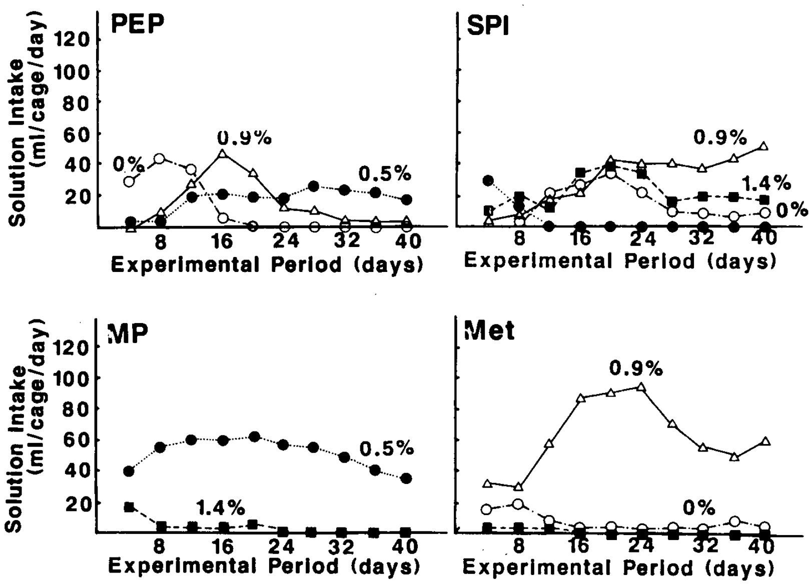

FIG. 2. The time course of preference for various concentrations of aqueous NaCl solutions in Wistar-Slc rats fed with four varietes of diet. Solution intakes are expressed as mean values for four days for each group of five rats. Deionized water, ---○--- ; 0.5% aqueous NaCl solution, ···●··· ; 0.9% aqueous NaCl solution, –△– ; 1.4% aqueous NaCl solution, --■-- ; 2.0% aqueous NaCl solution was not prefered for every group. Deionized water was not prefered for MP group. 0.5% aqueous NaCl solution was not prefered for Met group. 0.9% aqueous NaCl solution was not prefered for MP group. 1.4% aqueous NaCl solution was not prefered for PEP group.

TABLE 2. *The Sodium Contents and NaCl Conversion of Each Experimental Diets*

	PEP	MP	SPI	Met
Sodium contents (mg/100 g)	268	400	400	273
NaCl conversion (%)	0.68	1.02	1.02	0.69

Each experimental diets contained 98.5 (mg/100 g) Sodium, i.e. 0.25% NaCl from Harper's mineral mixture.

contents of SPI and Met were higher than those of PEP and MP. This might be caused by the contamination of sodium at a neutralization of isolated soybean protein. Fig. 3 shows the total NaCl intakes from various concentrations of aqueous NaCl solutions and each experimental diet in SHR and Wistar-Slc rats. In both the SHR and Wistar-Slc rats, the SPI and Met groups showed larger total NaCl intakes than the PEP and MP groups. When the NaCl was excluded from the experimental diets, this tendency did not change. The salt intake of SHR exceeded that of Wistar-Slc rats. Fig. 4 shows the compositions of plasma free amino acids. The contents of Val, Met, Ile, Phe and Lys, which are essential

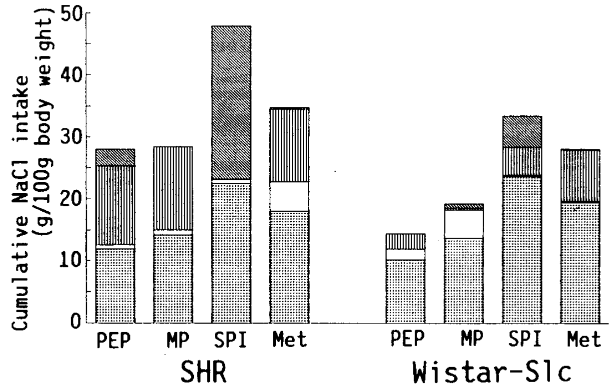

FIG. 3. Cumulative NaCl intakes from various concentration of aqueous NaCl solutions and each experimental diets in SHR and Wister-Slc rats. NaCl from each experimental diets, ▨; NaCl from 0.5% aqueous NaCl solution, □; NaCl from 0.9% aqueous NaCl solution, ▥; NaCl from 1.4% aqueous NaCl solution, ▧.

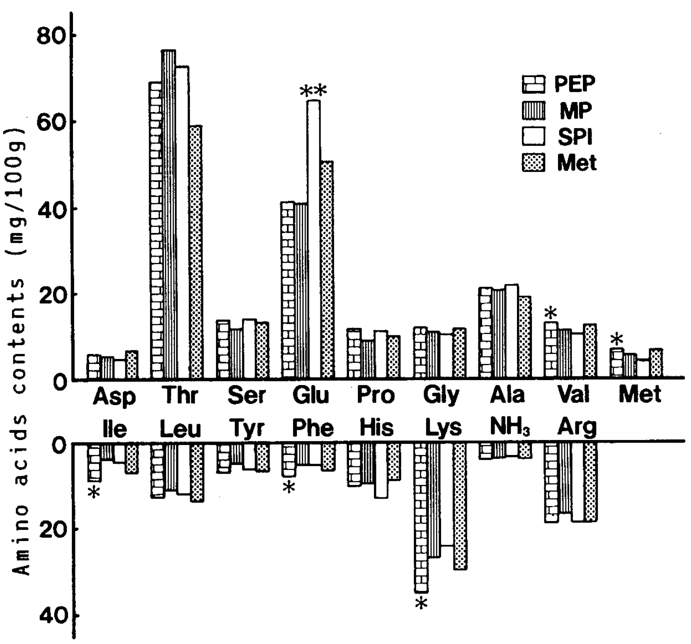

FIG. 4. Compositions of plasma free amino acids fed with four varietes of diets. PEP group, ⊟; MP group, ▥; SPI group, □; Met group, ▨; Significant difference from PEP; 0.01; **, from SPI; P<0.05; *.

amino acids, of the PEP group were significantly higher than those of the SPI group. However, the SPI group showed a significantly higher content of free glutamic acid compared with other groups.

⟨*Experiment 2*⟩

Fig. 5 shows the time course of preference for aqueous NaCl solutions and

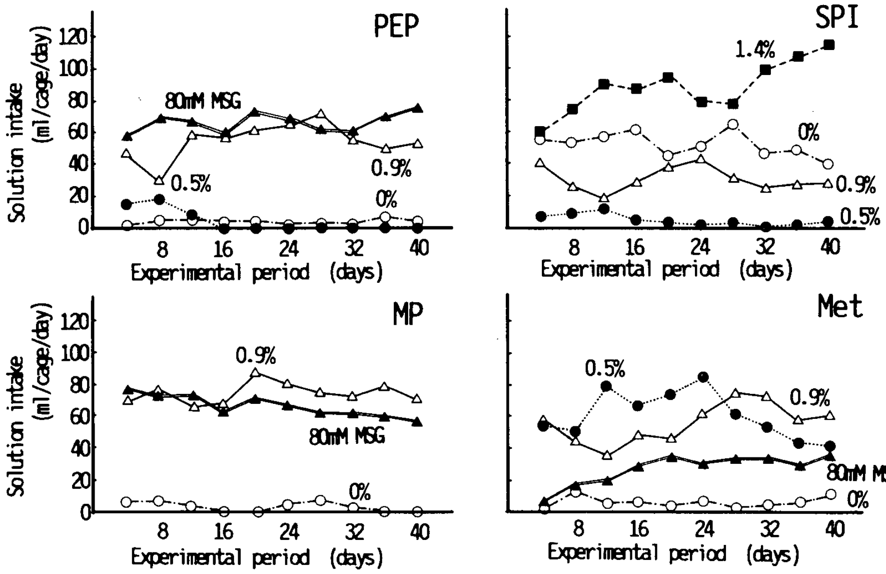

FIG. 5. The time course of preference for aqueous NaCl solutions and MSG solutions in SHR. Solution intakes are expressed as mean value for four days for each group of five rats. Deionized water, ---○---; 0.5% aqueous NaCl solution, ···●···; 0.9% aqueous NaCl solution, —△—; 1.4% aqueous NaCl solution, --■--; 80 mM MSG solution. =▲=; 2.0% aqueous NaCl solution, 20 mM MSG solution and 40 mM MSG solution were not prefered for every group. 0.5% aqueous NaCl solution was not prefered for MP group. 1.4% aqueous NaCl solution was not prefered for PEP group, MP group and Met group.

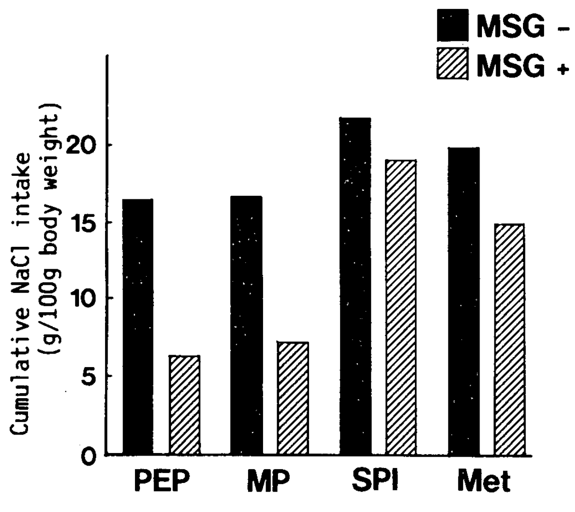

FIG. 6. Intake situations of aqueous NaCl solutions in SHR with and without the use of the MSG solutions, compared with Experiment 1. Cumulative NaCl intake without the use of MSG solutions, ■; cumulative NaCl intake with the use of MSG solutions, ▨.

MSG solutions in SHR. When the animals were allowed to select MSG solutions and aqueous NaCl solutions at the same time, the PEP group preferred the MSG solutions and thus the intake of the 0.9% aqueous NaCl solution decreased. The MP group also selected the MSG solutions. However, the SPI group did not select the MSG solutions and the Met group took little MSG solutions. This is similar to the salt taste preference of Experiment 1 wherein aqueous NaCl solutions were given alone. Regarding the concentration of the MSG solutions, the 80 mM MSG solution alone was selected in all of the PEP, MP and Met groups and none of the MSG solutions of lower concentration were selected. Fig. 6 shows the cumulative intake of aqueous NaCl solutions in SHR with and without the use of the MSG solutions, compared with Experiment 1. It was found that the SPI group did not change in the selection of aqueous NaCl solutions depending on the presence of the MSG solutions, while the PEP and MP groups preferred the 80 mM MSG solution. As a result, the intake of the aqueous NaCl solution of each concentration was decreased.

Discussion

The effect of protein sources, as one of the nutritional factors affecting salt taste preference, were examined. SHR fed with animal protein (i.e., PEP and MP) preferred low concentrated aqueous NaCl solutions and thus the salt intake was reduced, compared with those fed with vegetable protein (i.e., SPI). Though the salt taste preference of the rats fed with vegetable protein was lowered by improving the protein value of the vegetable protein (i.e., Met). It has been established that salt intake relates to the outbreak of hypertension (11). Epidemiological surveys of a hypertension incidence in japan indicate that salt, miso, processed soybean products and vegetables other than green and yellow ones are taken in large amounts while meat and egg are taken in small amounts (12). Based on these data, Kimura *et al.* found that the salt intake would be inversely proportional to the dietary protein level (13). They clarified that the animals fed with a low protein diet preferred an aqueous NaCl solution of higher concentration and thus the NaCl intake increased, compared with a high protein diet (14). Although the results of animal tests cannot be applied to man as such, it is considered that the present study supports the relationship between salt intake and protein intake. When the protein value of the vegetable protein was improved (i.e., Met), the preference for the aqueous NaCl solutions bacame similar to the one observed in the rats fed with animal protein. These facts suggest that the salt taste preference not only depends on genetic factors but also are modified with nutritional factors (i.e., dietary protein levels and dietary protein sources). Namely, it is considered that the salt taste preference is weakened when a well-balanced protein and the protein of amount sufficient for utilizing *in vivo* are taken, and that the NaCl intake is thus reduced.

When rats fed with animal protein were allowed to select MSG solutions together with aqueous salt solutions, the animals preferred the MSG solutions and the salt taste preference was further weakened. On the other hand, rats fed with vegetable protein never selected any MSG solution and the blood free glutamic acid was significantly higher than that of the rats fed with animal protein. This might be caused that the increase of blood free glutamic acid concentration would enhance the glutamic acid concentration in saliva and, as a result the threshold for MSG was elevated. In future, it would be necessary to investigate the glutamic acid concentration in saliva and the threshold for MSG of rats fed with vegetable protein. Following our previous report (7), it has been clarified that the turnover time of the taste bud cells of rats fed with a low-protein diet was longer than in the rats fed with a high-protein diet. Alternately on this experiment, it might be said that an insufficient amount of a protein from a nutritional viewpoint (for exsample, vegetable protein), prolonged the turnover time of the taste bud cells. In any case, it is suggested that the intake of animal protein would physiologically promote a reduction of salt intake and the coexistence of MSG would further enhance this effect.

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