Antihypertensive effect of chitosan in rats and humans

Hideo KATO,[a] Tomoko TAGUCHI,[a] Hiromichi OKUDA,*[b] Mari KONDO[c] and Minoru TAKARA[d]

[a] *Department of Food and Nutrition, Hiroshima Women's College*
[b] *2 nd Department of Medical Biochemistry and* [c] *2 nd Department of Pathology, School of Medicine, Ehime University*
[d] *Matsuyama Nishi Hospital*

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Abstract

The effect of dietary fibers on the hypertensive action of NaCl was examined by administration of a high salt diet containing alginic acid, which readily absorbs cations, or chitosan, which readily absorbs anions, to normotensive rats and SHRSP for 40 days. Addition of alginic acid to the high salt diet increased the amount of sodium and the addition of chitosan increased the amount of chloride in the feces of normotensive rats. Addition of chitosan to the high salt diet resulted in a significantly lower systolic blood pressure than addition of alginic acid in both groups. Serum ACE was significantly reduced in SHRSP fed with the high salt diet containing chitosan. Serum chloride ion was lower in the normotensive rats fed with the high salt diet containing chitosan than alginic acid.

In humans, the high salt diet increased the systolic blood pressure and serum ACE activity and chloride concentration after 1 h. and oral administration of chitosan inhibited these increases. It also reduced the serum bicarbonate level after 1 h, but did not affect the sodium concentration. Serum ACE in humans was found to be stimulated by chlorideion.

These results suggest that chitosan prevents increase in the systolic blood pressure of humans induced by high salt intake by inhibiting intestinal absorption of chloride, an activator of ACE. Based on these results, the relationship between serum ACE and chloride concentration was discussed.

Key words dietary fiber, chitosan, chloride, hypertension.

Abbreviations SHRSP, stroke-prone spontaneously hypertensive rats ; ACE, angiotensin converting enzyme.

Introduction

A high level of sodium chloride in the diet has been shown to increase the blood pressure of humans and animal models of hypertension.[1-3] Moreover, restriction of dietary NaCl decreased the blood pressure and its subsequent supplementation increased the blood pressure.[4-8] However, it is unknown which ion (sodium or chloride) in NaCl is responsible for modulation of the blood pressure.

In 1904, Ambarad and Beaujard suggested that increased retention of chloride might be important in the pathogenesis of hypertension.[9] But in 1954, Dahl and Love proposed the now widely accepted view that sodium ion alone cause hypertension.[10] This view is based on the following observations. First, after decrease in the blood pressure by restricition of dietary sodium chloride, supplementation of dietary chloride without sodium (ammonium chloride) failed to increase the blood pressure. Second, after increase in the blood pressure by supplementation of dietary sodium chloride, blood pressure decreased again on restricting sodium alone (by substituting ammonium chloride for sodium chloride).

On the other hand, several investigators noted that the ingestion of nonchloride sodium salts, such as sodium carbonate, sodium ascorbate and sodium

*〒 791-02 愛媛県温泉郡重信町志津川
愛媛大学医学部医化学第二 奥田拓道
Shigenobu, Onsen-gun, Ehime 791-02, Japan

citrate, did not cause an elevation of the blood pressure in animal models or humans.[11,12] Moreover, ingestion of chloride salts, such as calcium chloride, potassium chloride, choline chloride and lysine chloride, rather than nonchloride salts, has been shown to be associated with elevation of the blood pressure.[13,14]

A problem in all these previous experiments is that substitutions of other salls such as ammonium chloride, choline chloride and lysine chloride for sodium chloride cause various side effects such as acidosis and reduction of weight gain. Therefore, conditions not involving these substitutions are required to clarify the roles of sodium and chloride in regulating the blood pressure. Accordingly, in this study we used the dietary fibers alginic acid, an anionic fiber that absorbs sodium ion, and chitosan, a cationic fiber that absorbs chloride ion, to determine which ion (sodium or chloride) in NaCl is responsible for elevation of the blood pressure.

Materials and Methods

Materials : Chitosan used in this experiment was 81% deacetylated chitin which was kindly provided by Ueno Fine Chemicals Industry LTD.

Animals and treatments : Male SHRSP and age-matched Wistar rats (normotensive rats) were housed individually in suspended stainless steel cages in a room maintained at 24±2°C with a 12 h light : dark cycle and given commercial nonpurified diet (MF, Oriental Yeast, Tokyo, Japan) until 5 weeks of age. Each strain was then divided into two groups (5-6 rats/group) and given a basal diet supplemeted with 3 % NaCl with or without 5 % alginic acid or 5 % chitosan for 40 days (Table I).

Food was available from 5 : 00 p.m. to 9 : 00 p.m. and deionized water was available continuously. The systolic blood pressure of conscious, prewarmed restrained animals was measured by the tail-cuff method with an electro-sphygmomanometer and physiograph recorder (model PE-300 ; Nacro Biosystems, Houston, TX). Results by this indirect method correlate closely with those obtained by direct measurement of the arterial blood pressure. The body weights and food intakes of all animals were recorded throughout the experimental period.

Assay of ACE in serum[15] : Serum (20 μl) was incubated with 100 μl hippuryl-glycyl-glycine solution (pH 8.0) consistiong of Hepes buffer containing 400 mM Na_2SO_4 for 30 min. Then the mixture was deproteinized by adding Na-tungstate plus 0.33 M H_2SO_4, the glicyl-glycine liberated was treated with borate-buffer (pH 9.6) containing trinitrobenzene sulfonate and the absorbance at 420 nm was measured against a serum blank. One unit of ACE activity was defined as the amount releasing 1 μmol of hippuric acid per min per liter of serum at 37°C. In this assay, chloride ion in the reaction mixture was derived only from serum.

Measurement of serum chloride level : 1.2 ml of serum were mixed with 0.8 ml of H_2O, 0.5 ml of 2 % Ba $(OH)_2$ and 0.5 ml of 2 % $ZnSO_4$ and centrifuged. The supernatants were titrated with 0.02N Hg $(NO_3)_2$ after addition of 0.1% diphenylcarbazone as indicator.

Measurements of fecal sodium and chloride contents : Five normotensive rats fed the experimental diet is shown in Table I. The feces of each rat were collected for 24 h periods from 9 : 00 a.m., dried at 105°C for 24 h and ashed at 600°C for 3 h. For measurement of sodium, 0.1 g of the ash was suspended in 10 ml of 1.5N HCl and centrifuged. Sodium in the supernatant was then measured with an atomic absorption apparatus after 10-to 20-fold dilution with 0.5 N HCl. For measurement of chloride, 0.1 g of the ash was suspended in 5 ml of 0.1 N NaOH and centrifuged, and chloride

Table I Compositions of experimental diets.

Ingredient	Alginic acid diet	Chitosan diet
	g/kg	
Sucrose	145	145
Casein[1]	240	240
AIN 76TM vitamin mix[1]	20	20
Choline chloride	0.1	0.1
AIN 76TM mineral mix[1]	40	40
NaCl	32	32
Soybean oil[2]	20	20
Alginic acid[3]	50	0
Chitosan (10B)[4]	0	50
Corn starch	452.9	452.9

[1]From Oriental Yeast, Tokyo, Japan.
[2]From Nacalai Tesque, Kyoto, Japan.
[3]From Sigma Chemical Co., St Louis, USA.
[4]From Katokichi Co., Kagawa, Japan.

in the supernatant was measured by the method of Schales and Schales.[16]

Experiments on humans : Seven healthy men, 23-27 years old, who agreed to participate in this experiment, came to Matsuyama Nishi Hospital before breakfast on June 21, 1992. Arterial blood was drawn from the brachial artery and then at 8 : 30 a.m. they were given a breakfast of 1099 kcal containing 13.3 g of NaCl, consisting of steamed rice, miso-soup, boiled eggplant, potatoes, carrots, soybean cake, short-necked clam, salty saurel and pickled radish. Sanples of arterial blood were taken before, and 1 and 3 h after breakfast. These blood samples were centrifuged at 3000 × g for 30 min and the chloride, carbonate, sodium and ACE contents of the sera separated were measured. Carbonate ion was measured by the method of Singer and Hestings[17] and sodium ion by the method of Kilgariff and Owen.[18]

Blood pressure was also measured before and after breakfast. The same subjects came again to Matsuyama Nishi Hospital at 8 : 00 a.m. before breakfast one week later. Arterial blood was taken from each subject and their blood pressure was measured. Just after eating the same breakfast containing 13.3 g of NaCl at 8 : 30 a.m., each subject took 5 g of chitosan powder. Collection of arterial blood and measurement of the blood pressure were carried out before and, 1 and 3 h after breakfast, and the chloride, carbonate, sodium and ACE contents of serum samples were determined.

Statistical analyses : Data on blood Pressure were analyzed by the Newman-Keuls test and other data by Student's *t* test.

Results

Experiments on rats

As shown in Table II, alginic acid caused significantly more sodium excretion than chitosan (7.00 ± 1.38 mg/day/rat vs 1.38±0.18 mg/day/rat), whereas chitosan caused higher excretion of chloride than alginic acid (9.60±2.08 mg/day/rat vs 1.20±0.40 mg/day/rat). In the absence of these dietary fibers, sodium and chloride excretions in the feces were found to be 0.39 ± 0.07 mg/day/rat and 0.28 ± 0.07 mg/day/rat, respectively. Then, normotensive rats and SHRSP were given a diet containing alginic acid or chitosan for 40 days. There were no significant differences in weight gains on the two diets of either normotensive rats or SHRSP (Fig. 1).

Table II Effect of dietary fibers on chloride and sodium excretions in feces of rats. Five normotensive rats were given the experimental diets shown in Table I for 5 days. Feces were collected for 24 h periods from 9 : 00 a.m. Data are means±S.E..

	Fecal excretion (mg/day/rat)	
	Sodium	Chloride
Alginic acid diet	7.00±1.38	1.20±0.40
Chitosan diet	1.38±0.18	9.60±2.08
	$p<0.01$	$p<0.02$

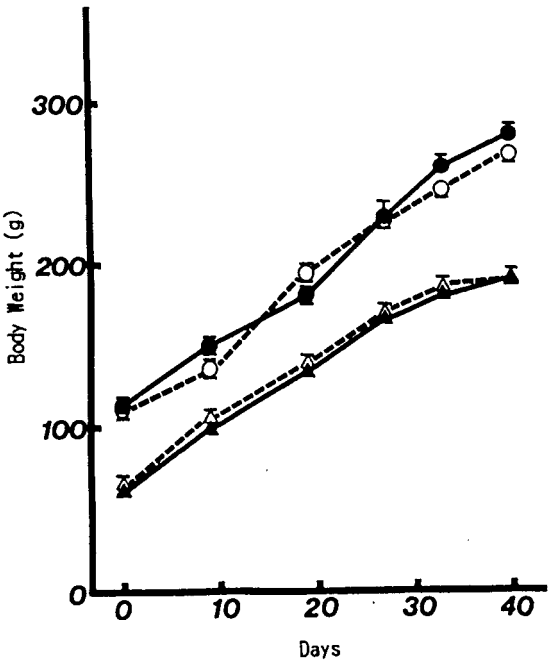

Fig. 1 Body weight changes during the experimental period. All rats weighed at the same time before feeding. ○, ● : normotensive rats ; △, ▲ : SHRSP ; ○, △ : alginic acid diet ; ●, ▲ : chitosan diet. Data are means±S.E. for 5 rats.

Serum chloride and ACE activity, and the systolic blood pressure of both normotensive rats and SHRSP were estimated after administration of alginic and/or chitosan diet for 40 days. Serum chloride level was compared between the rats fed with alginic acid and chitosan diet (Fig. 2). In normotensive rats, serum chloride level reduced significantly with the chitosan diet as compared with the alginic acid diet. In SHRSP,

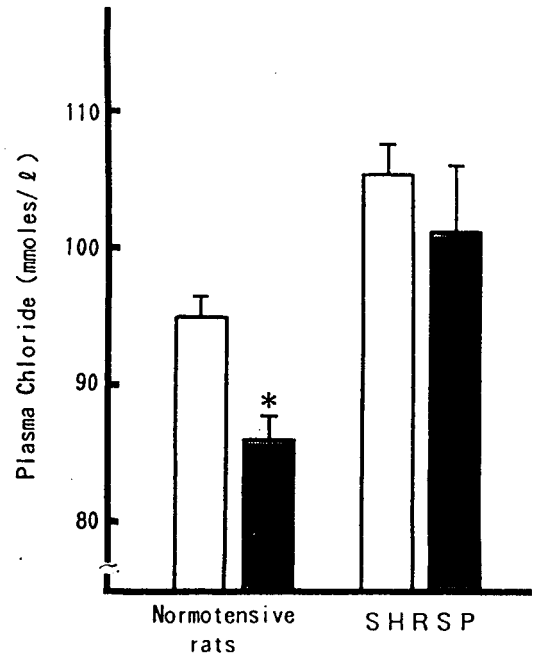

Fig. 2 Effect of dietary fibers on serum chloride in rats.
□ : Alginic acid diet ; ■ : chitosan diet. Data are means ±S.E. for 5 rats. * significant difference ($p<0.05$) from the value with alginic acid diet in normotensive rats.

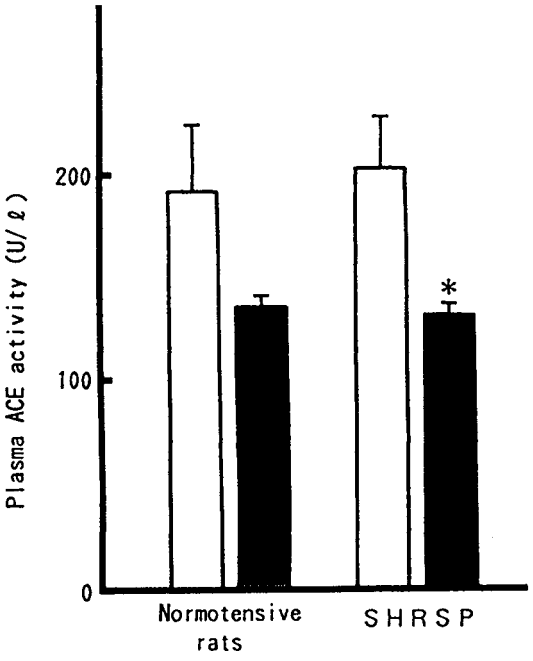

Fig. 3 Effect of dietary fibers on the serum angiotensin converting enzyme activity in rats.
□ : Alginic acid diet ; ■ : chitosan diet. Data are means ±S.E. for 5 rats. * significant difference ($p<0.05$) from the value with alginic acid diet in normotensive rat.
* significant difference ($p<0.05$) from the value with alginic acid diet in SHRSP.

serum chloride level did not reduce significantly in the rats fed with the chitosan diet. The chitosan diet, however, caused significant reduction of serum ACE activity in SHRSP (Fig. 3). On the other hand, in normotensive rats, serum ACE level was not decreased significantly in the rats fed with the chitosan diet. Although correlation between chloride level and ACE activity in serum failed to be demonstrated, administration of the chitosan diet resulted in significantly lower systolic blood pressures in both groups than administration of the alginic acid diet ($p<0.01$ in both groups), as shown in Fig. 4.

Experiments on human

Next we carried out experiments on humans to prove that chloride in dietary sodium chloride partisipated in hypertension via elevation of ACE activity, because the time of eating can be strictly regulated in humans but not in experimental animals.

Seven healthy men were given a breakfast containing 13.3 g of NaCl as described in "Materials and Methods". One hour after breakfast, their systolic

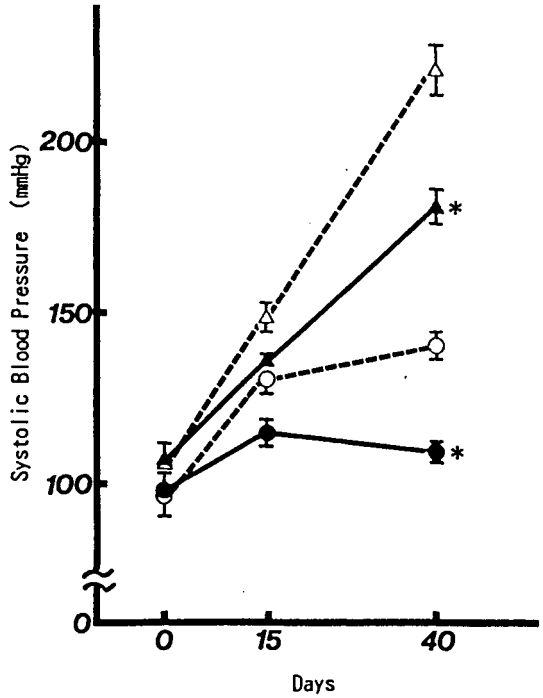

Fig. 4 Effects of alginic acid and chitosan diets on systolic blood pressure of normitensive rats and SHRSP.
○, ● : normotensive rats ; △, ▲ : SHRSP ; ○, △ : alginic acid diet ; ●, ▲ : chitosan diet. Data are means±S.E. for 5 rats.
* significant difference ($p<0.01$) from the value with alginic acid in normotensive rats and SHRSP.

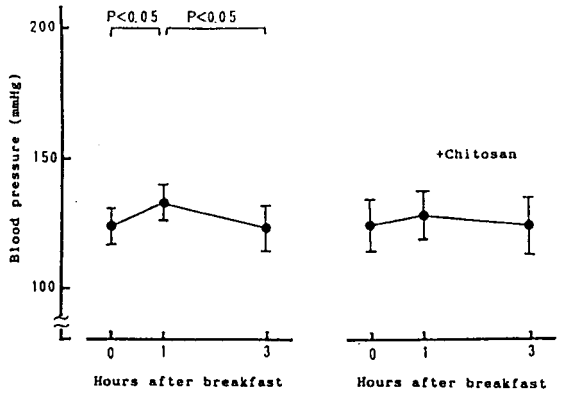

Fig. 5 Effect of chitosan on the systolic blood pressure of humans. Seven healthy men were fed a breakfast containing 13.3 g of NaCl, and the same breakfast with 5 g of chitosan one week later, as described in "Materials and Methods". Data are means±S.E..

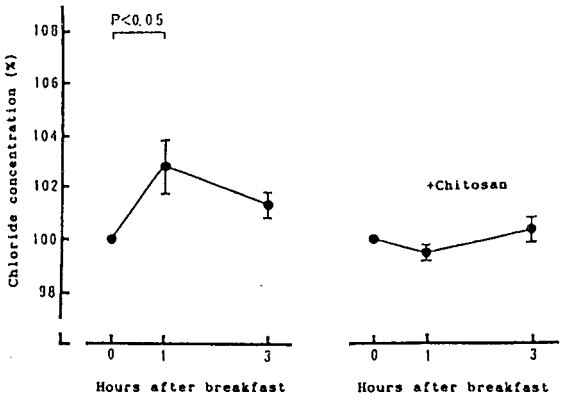

Fig. 7 Effect of chitosan on the chloride concentration of human serum. Values are relative to that (102 mM) before breakfast. Experimental procedures were as described in "Materials and Methods" and the legend for Fig. 5. Data are means±S.E..

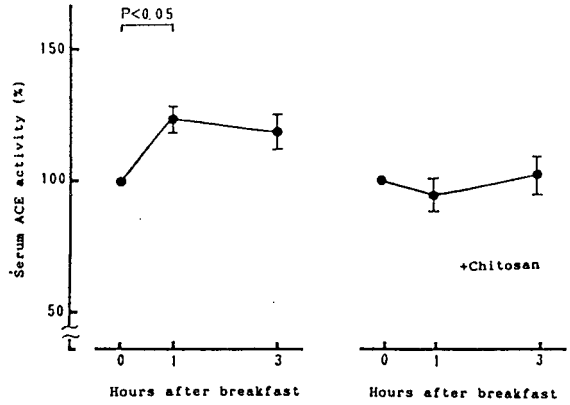

Fig. 6 Effect of chitosan on ACE activity in human serum. Activity is shown relative to that (230 U/l) before breakfast. Experimental procedures were as described in "Materials and Methods" and the legend for Fig. 5. Data are means±S.E..

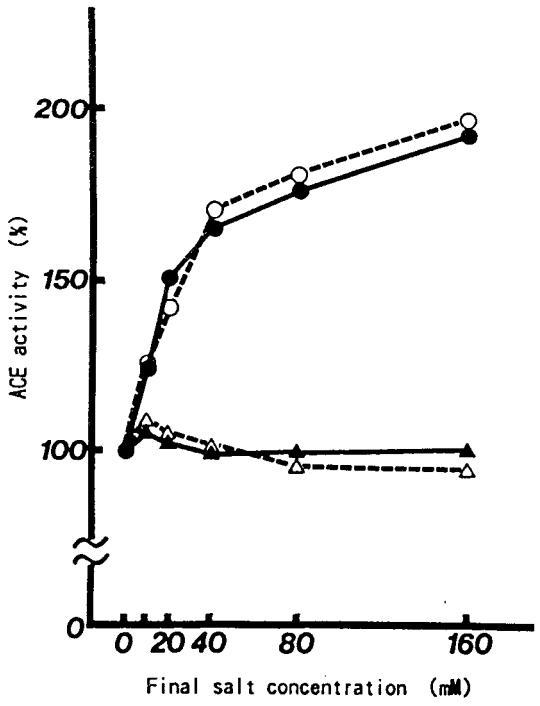

Fig. 8 Activation of ACE in human serum by various salts. Activities are shown as percentages of that without additional salts (172 U/l).
Serum (20 μl) was preincubated with 20 μl of H_2O or various salt solutions for 5 min at 37°C. ACE activity was measured by adding 100 μl of substrate solution. ○ : NaCl ; ● : KCl ; △ : CH_3COONa ; ▲ : $NaHCO_3$. Values are means for two separate experiments.

blood pressure was significantly elevated and returned to the initial level after 3 h. On the other hand, their systolic blood pressure did not increase after breakfast with chitosan (Fig. 5). The serum ACE activity was elevated 1h after breakfast without, but not with, chitosan (Fig. 6). In addition, their serum chloride was significantly elevated 1 h after breakfast without, but not with chitosan (Fig. 7). Increase in serum chloride was associated with reduction of serum bicarbonate, which decreased from 24 mM to 22.5 mM 1 h after

breakfast without chitosan returned to the original level 3 h after the meal. No reduction of serum bicarbonate was observed after breakfast with chitosan. The serum sodium level did not change after breakfast with or without chitosan. Finally, serum ACE in humans was found to be stimulated by NaCl or KCl but not by CH_3COONa or $NaHCO_3$, as shown in Fig 8. The result indicated that the ACE was activated by chloride ion but not by sodium ion.

Discussion

The present experiments were carried out to clarify which ion (sodium or chloride) in NaCl is important for modulation of the blood presuure.

First, male normotensive rats and SHRSP were given diets containing 3 % NaCl, and 5 % alginic acid or 5 % chitosan (Table. I). These diets both contained the same amounts of sodium and chloride. The sodium content of the feces of rats fed the diet containing alginic acid was higher than that of rats given the diet containing chitosan and conversely, the chloride content of the feces of rats given diet containing chitosan was higher than that of those given alginic acid. Thus chitosan enhanced chloride excretion in the feces whereas alginic acid enhanced sodium excretion in the feces (Table II). These diets did not affect the weight gains of normotensive rats SHRSP (Fig. 1). However, the chitosan diet resulted in a lower systolic blood pressure than the alginic acid diet in both strains of rats (Fig. 4).

A question arises as to what is the mechanism of reduction of blood presure due to the chitosan diet. Although chitosan increased clearly chloride excretion in feces, serum chloride in SHRSP did not reduce significantly. On the other hand, serum ACE activity decreased significantly in these rats. In contrast to SHRSP, serum chloride level decreased significantly in normotensive rats, whereas serum ACE activity did not reduce significantly in these rats. These inconsistent results may come from the time of estimation of serum chloride and ACE activity. We could not obtain clear correlation among systolic blood pressures, serum chloride and ACE levels in animal experiments.

Based on these results in experimental animals, we carried out experiments on humans to clarify this correlation. Seven healthy men were given breakfast containing 13.3 g of NaCl at 8 : 30 a.m., and one week later were given the same breakfast with 5 g of chitosan at the same time. Before, and 1 and 3 h after the breakfast, we measured their systolic blood pressures and took arterial blood samples for measurements of serum levels of ACE activity, chloride, bicarbonate, and sodium. Results showed that the systolic blood pressure, and serum ACE activity and chloride increased simulaneously 1 h after breakfast without chitosan, but not after breakfast with chitosan (Fig. 5. 6 and 7). The elevation of serum chloride was associated with reduction of serum bicarbonate. This seems to reflect the constancy of anionic ions in the serum. In contrast to chloride and bicarbonate, the sodium level in the serum was maintained at the same level after breakfast without chitosan as that before the meal.

Serum ACE in humans was activated by chloride ion but not by sodium ion as shown in Fig 8. The result suggests that elevation of blood pressre by NaCl may be induced by activation of ACE through increase in serum chloride and chitosan prevents increase in the systolic blood pressure by inhibiting intestinal absorption of chloride. In the present experiment on humans, serum ACE activity was elevated from 230 U/l to 280 U/l with concomitant increase in serum chloride from 102 mM to 105 mM.

In the assay of serum ACE in Fig. 8, the ACE was preincubated with various salt solutions, and diluted 3.5 times with the substrate solution. The ACE activity was estimated as a function of final concentrations of added salts. Therefore, we can not deduce correctly the relationship between serum ACE activity and chloride levels from the results of Fig. 8. In order to clarify the relationship, it is necessary to examine the effect of chloride concentrations in preincubation of ACE assay on the enzyme activity, because chloride concentrations in the preincubation corresponds to serum chloride levels.

In Fig. 9, ACE activities in human serum were estimated as a function of chloride concentrations in preincubation of its assay. According to the result in Fig. 9, 102 mM and 105 mM of serum chloride ion were corresponded to 212U/l and 222U/l of ACE activities, respectively. Thus, an increase in chloride ion from 102 mM to 105 mM caused elevation of 10 U/l of ACE.

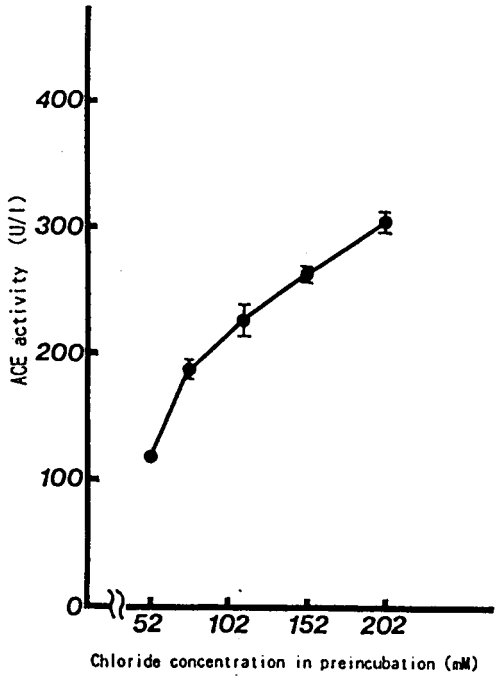

Fig. 9 Effect of chloride concentrations in preincubation on serum ACE activity.
Initial of serum ACE activity and chloride level are 118 U/l and 104 mM, respectively. Serum (20 μl) was preincubated with 20 μl of H_2O or various chloride (KCl, NaCl) solutions for 5 min at 37°C. ACE activity was measured by adding 100 μl of substrate solution. Data are means±S.E. (n=5).

In the present experiment on humans, however, serum ACE activity was elevated from 230 U/l to 280 U/l. Although increase in serum chloride from 102 mM to 105 mM contributed partly to the elevation of serum ACE activity, the increment of 50 U/l (280 U/l-230 U/l) of ACE activity could not be explained solely by the increase in serum chloride.

Two possible mechanisms will be postulated for elevation of serum ACE activity. One is that serum chloride level may be elevated higher prior to 1 h after breakfast than at 1 h after the meal (Fig. 7). The other is that serum ACE may be activated by other factors than chloride ion.

Experiments are now in progress to clarify these points.

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和文抄録

食塩中の Na^+ と Cl^- のうち，いずれが血圧上昇に関与しているかを明らかにする実験を，ラット及びヒトで行った。

まず，正常ラットを用い，高塩食（32 g NaCl/kg）と共にアルギン酸を経口投与すると，糞中の Na^+ が多く排泄され，キトサンを高塩食と共に投与すると，Cl^- が糞中に多く排泄されることを確認した。次に，正常血圧ラット及び自然発症高血圧ラットに高塩食を 40 日間投与したところ，両ラット共，アルギン酸投与群に比べてキトサン投与群で有意に収縮期血圧が低下することが明らかになった。

次に,健康な男性 7 名について 13.3 g の NaCl を含む高塩食，及び高塩食とキトサン 5 g を食した前後の血圧，及び動脈血 Na^+，Cl^-，ACE 等について測定した。高塩食を摂取し，1 時間後に収縮期血圧の上昇，Cl^- の上昇，ACE の上昇，重炭酸イオンの減少が認められたが，Na^+ には変化は認められなかった。又，高塩食と共にキトサンを摂取した場合には，血圧，Cl^-，ACE，重炭酸イオンに有意な変動は認められなかった。又，Cl^- はアンジオテンシン転換酵素（ACE）を活性化することから，NaCl の中の Cl^- が ACE の上昇を介して，血圧の上昇を引き起こす可能性について考察した。

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