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Anti-Bradykinin Activity Found in Beet (*Beta vulgaris* L. var. *rapa* Dumort. f. *rubra* DC.)

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The potent anti-bradykinin activity was found in the beet root; 4—7 μg of bradykinin was antagonized by the crude beet juice equivalent to 1 g of the root in the assay of contraction of the guinea-pig ileum. This substance was quite stable under heat treatment at 100° for 30 min, and its molecular weight seemed to be around 1000.

The bradykinin antagonist was purified as follows: the grated beet roots were lyophilized and then extracted with boiling 99% ethyl alcohol. After removal of the alcohol, the residue was dissolved in water and treated with ether. The aqueous layer was concentrated and applied to a Sephadex G-15 column and a Bio-Gel P-2 column successively. The active fraction obtained by these procedures was developed as a single spot on a silica gel thin-layer chromatogram.

The inhibitory effects of this substance were observed on the smooth muscle contraction of guinea-pig, the vasodilatation in dog and the increase in the capillary permeability in rat, all of which were induced by bradykinin. The carrageenin-induced edema in the rat's paw and some of the above biological responses by histamine and 5-hydroxytryptamine were also suppressed distinctly or slightly by the beet antagonist.

Bradykinin, one of the physiologically active peptides kinins, is considered to play important roles in numerous pathological processes, but the roles have not been completely clarified. Effective foreign substances depressing the bradykinin activity, therefore, would be of value in controlling the pathological reactions induced by the bradykinin formed endogeneously and in elucidating its physiological significance. Many studies on bradykinin antagonists *in vivo* or *in vitro* have been reported as one of the ways for such purposes. Stewart and Woolley[2] reported the inhibitory actions of bradykinin analogues upon the effect of bradykinin on the rat uterus; however, all of those substances were accompanied by a bradykinin-like activity in higher concentration. This characteristic seems to limit the usefulness as bradykinin antagonists in any *in vivo* situation. Certain anti-histamine compounds, particularly phenothiazine derivatives,[3] demonstrated anti-bradykinin activity when they were tested on the isolated guinea-pig ileum. Some thiaxanthene and dibenzocycloheptane derivatives also exerted powerful anti-bradykinin activity *in vitro*,[4] however an inhibitory activity *in vivo* was difficult to be demonstrated. Collier and Shorley examined analgesic antipyretic drugs such as acetylsalicylic acid, phenylbutazone, amidopyrine and phenazone,[5] and mefenamic and flufenamic acids,[6] which had a high potency as bradykinin antagonists on bronchoconstriction of guinea-pig *in vivo*. Nevertheless, most of them did not particularly antagonize the actions of bradykinin on the capillaries of guinea-pig *in vivo*, on the guinea-pig

1) Location: *12, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo.*
2) J.M. Stewart and D.W. Woolley, "International Symposium on Vaso-Active Polypeptides: Bradykinin and Related Kinins," ed. by M. Rocha e Silva and H.A. Rothschild, III International Pharmacological Congress and Soc. Bras. Farmacologia e Terapêutica Experimental, São Paulo, 1967, p. 7.
3) J.D. Horowitz and M.L. Mashford, *J. Pharm. Pharmacol.*, **21**, 51 (1969).
4) J. Garcia Leme and M. Rocha e Silva, *Brit. J. Pharmacol.*, **25**, 50 (1965).
5) H.O.J. Collier and P.G. Shorley, *Brit. J. Pharmacol.*, **15**, 601 (1960).
6) H.O.J. Collier and P.G. Shorley, *Brit. J. Pharmacol.*, **20**, 345 (1963).

ileum or the rat duodenum *in vitro*. It appears difficult to find satisfactory bradykinin antagonists both *in vivo* and *in vitro* situations among the compounds reported to date.

Meanwhile, a potent anti-bradykinin activity was originally found in the root of beet (*Chenopodiaceae*, *Beta vulgaris* L. var. *rapa* DUMORT. f. *rubra* DC.) at the authors' laboratory. The authors had interest in this anti-bradykinin substance, because it might help to study the physiological roles of kinins or it might be therapeutically useful in some pathological phenomena related to kinins.

In the present paper, the authors described the methods to purify the antagonist from beet roots and its inhibitory effects on some biological activities of bradykinin both *in vitro* and *in vivo* experiments such as: contraction of the guinea-pig ileum, increase in the blood flow due to vasodilatation and increase in the capillary permeability. Inhibitory effects on histamine and 5-hydroxytryptamine and that against carrageenin-induced inflammation were also described, and the effect on anaphylactic shock was referred.

Material and Method

Materials——Fresh beet roots, harvested in Nagano Prefecture, Japan, during the months of May and June, were purchased from a grocery store.

The following chemical materials and reagents were used: synthetic bradykinin (Protein Research Foundation, Minoh, Osaka); histamine dichloride (E. Merck AG); 5-hydroxytryptamine creatinine sulfate (5-HT, Daiichi Pure Chemicals Co., Ltd.); pontamine sky blue 6B (Tokyo Kasei Kogyo Co., Ltd.); carrageenin (Seakem 402, Marine Colloids Inc.); Sephadex G-15 and G-50 (Pharmacia Fine Biochemicals); Bio-Gel P-2 (Bio-Rad Laboratories); Silica Gel H acc. to Stahl (E. Merck AG). Other chemicals were of guaranteed reagents.

Assay Method of Bradykinin Antagonist——An assay of bradykinin antagonist was performed on the isolated guinea-pig ileum. A strip from 2 to 3 cm long was suspended in 10 ml of Mg^{2+}-free Tyrode solution at 30° in an organ bath. The contractile responses of the various concentrations of synthetic bradykinin for 45 sec after injection were detected by a force displacement transducer (SB-1T, Nihon Kohden) attached to an electronic manometer (MP-3A, Nihon Kohden) and a DC amplifier (AD 2-22, Nihon Kohden), and were recorded on an ink writing oscillograph (WI-130, Nihon Kohden). At the beginning of assay, responses to a series of four doses of synthetic bradykinin (usually 20, 40, 60, and 100 ng, dissolved in physiological saline) were checked, and each of them was repeated two or three times to obtain a standard dose-response curve.[7a] Samples (0.1—0.2 ml) to be tested as an antagonist were infused into the organ bath 1 min before the addition of 100 ng bradykinin. In order to get reasonable and accurate estimation of inhibition, after a few preliminary trials, several stages of sample concentration were made with saline and examined so as to observe the inhibition around 50%, and then the reasonable inhibitory activity was calculated out from the 50% inhibition. Inhibitory activities against histamine and 5-HT were also assayed in the same manner.

Vasodilatation——The vasodilating activity of bradykinin was determined by measuring the increase in blood flow according to the method of Moriya, *et al.*[7] A dog weighing about 10 kg was anesthetized with an intraperitoneal administration of sodium pentobarbital (Abbott Lab.). Cannulae attached to a pressure transducer (MF-2T, Nihon Kohden) were inserted into the femoral artery, and the blood flow was detected by a magnetic flow meter (MF-2, Nihon Kohden), being recorded through the DC amplifier on an auto-writing oscillograph (WI-180U, Nihon Kohden).

Capillary Permeability——Wistar albino rats weighing 200—250 g of either sex were anesthetized intraperitoneally with sodium pentobarbital in a dose of 50 mg/kg, and then clipped of their venter fur by treating with a depilatory, from the venter cranialis to the venter caudalis. According to the method of Bhoola, *et al.*,[8] the rats were injected intravenously with 5% (w/v) solution of pontamine sky blue 6B in saline *via* the femoral vein in a dose of 1.2 ml/kg. Intradermal injections of the permeability increasing agents in saline were then made in 0.1 ml volumes, and 30 min later, the diameter of the blue lesion caused by the leakage of the circulating dye at the site of injection was measured.

To test the inhibitory effect of the beet extract, the following procedures were adopted. At first, the areas of blue spots induced by various concentrations of bradykinin and histamine were recorded as described above. The animal was then injected with the sample G[9] (1 ml/kg) *via* another femoral vein. Five minutes

7) *a*) C. Moriwaki, Y. Hojima, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **22**, 975 (1974); *b*) H. Moriya, K. Yamazaki, and H. Fukushima, *J. Biochem.* (Tokyo), **58**, 201 (1965).
8) K.D. Bhoola, J.D. Calle, and M. Schachter, *J. Physiol.* (London), **152**, 75 (1960).
9) One milliliter of the sample G (see Result) antagonized 10 μg of bradykinin when its potency was assayed on the isolated guinea-pig ileum.

later the same amount of each agonist was injected intradermally on the other side of the venter. After 30 min, the size of these lesions was measured and compared with those obtained from the same animal before treatment with the sample G.

Anti-inflammatory Test——Two groups of 15 Wistar albino rats weighing 120—160 g were used. The animals were anesthetized with ether sufficiently deep to ensure that the limbs were flaccid. Edema was induced by the injection of 0.1 ml of 1% (w/v) carrageenin dissolved in saline beneath the plantar aponeuroses of the hind paws. Thirty minutes before the injection of carrageenin, the sample G or saline for the control were administered intraperitoneally in a dose of 5 ml/kg. The swelling degrees of the rat hind paws were measured by the modified apparatus of Harris and Spencer.[10]

Result

Preliminary Experiments

The fresh beet roots (100 g) grated with a Japanese radish grater, were suspended in 100—150 ml of saline (0.15 M, NaCl) and stirred vigorously for 30 min. The crude beet juice was squeezed from the homogenate through two layers of cotton cloth. The juice was centrifuged, and the supernatant was adjusted to pH 7.4 with 1 N NaOH. Its volume was finally adjusted to 200 ml with saline for use in the following preliminary experiments. The absorbance of the clear juice at 280 nm (a Hitachi spectrophotometer, model 124) was 40—50 A_{280}/ml.

The inhibitory activity of the beet juice (diluted in several stages) against bradykinin (100 ng) was assayed on the isolated guinea-pig ileum. Approximately 4—7 μg of bradykinin were usually antagonized by the beet juice equivalent to 1 g of the fresh beet root.

The juice was diluted 5 times (J×5) with saline. The mixtures of 80 ng of bradykinin (0.4 ml) and 0.2 ml of J×5 were incubated for 1, 2, 5 and 11 min at 30°. Each mixture was assayed on the guinea-pig ileum. There were no significant changes in the inhibitory action of the beet juice due to the incubation period (A of Fig. 1). The beet juice was heated

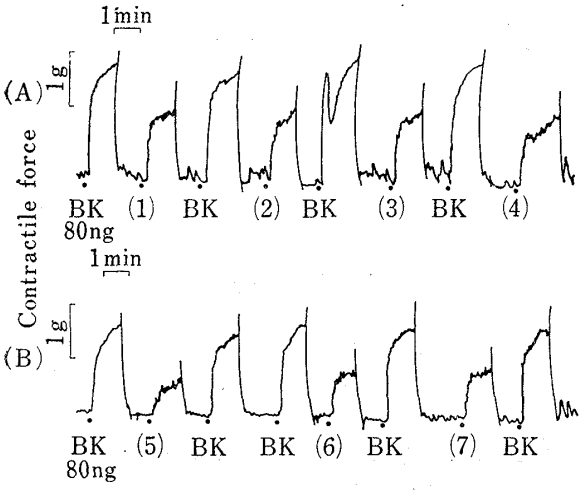

Fig. 1. Inhibitory Action of the Beet Juice on the Bradykinin Effect upon the Guinea-Pig Ileum

(A): 80 ng of bradykinin (0.4 ml) was incubated with 0.2 ml of the beet juice (5 times diluted, J×5) at pH 7.4, 30° for the following minutes separately. (1), 1 min; (2), 2; (3), 5; (4), 11

(B): Each 0.2 ml of the heated juice (5 times diluted, HJ×5), non-heated juice (J×5) and the heated, 10 times diluted juice (HJ×10) was incubated with 80 ng of bradykinin (0.4 ml) at pH 7.4, 30° for 1 min. (5), HJ×5; (6), J×5; (7), HJ×10

BK: bradykinin

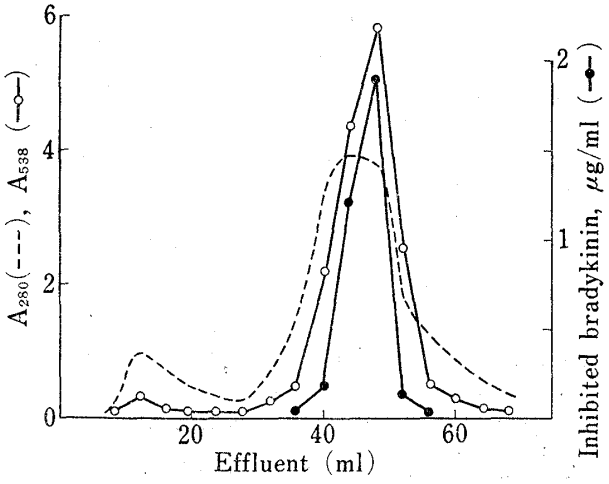

Fig. 2. Gel Filtration of the Beet Juice on a Sephadex G-50 Column

sample, 2 ml of the beet juice adjusted to pH 7.4; column size, 1.5×40 cm; buffer, 0.05 M phosphate buffer, pH 7.4, containing 0.05 M NaCl

10) J.M. Harris and P.S.J. Spencer, *J. Pharm. Pharmacol.*, **14**, 464 (1962).

at 100° for 30 min, and then the heated juice was diluted 5 times (HJ×5) and 10 times (HJ×10) with saline. Eighty nanograms of bradykinin was incubated with 0.2 ml of each sample, HJ×5, J×5 or HJ×10, and assayed (B of Fig. 1). The inhibitory activity of the juice to bradykinin remained stable. On the other hand, the bradykinin inactivating enzymes found in other plants at the authors' laboratory[11] were completely inactivated under the heat treatment. Further it was interesting that the inhibitory activity of the juice seemed to be slightly elevated by heating (B of Fig. 1). Therefore, it was recognized that the mode of the inhibitory action of the beet juice was not enzymatic but antagonistic.

The beet juice was applied to a Sephadex G-50 column and eluted with 0.05M phosphate buffer (pH 7.4, containing 0.05M NaCl). The fractions of the eluate were checked for their optical density for convenience at 280 nm, and 538 nm (λ_{max} of betanin),[12] and assayed for their inhibitory activities against bradykinin. The activity pattern coincided with the absorbance pattern of the red-violet pigment betanin (Fig. 2). However, the partially purified betanin from the beet root by the authors according to the method of Pucher *et al.*[13] had no inhibitory activity against bradykinin on the guinea-pig ileum. So the active substance would differ from betanin, but seemed to have a similar molecular weight to that of betanin,[14] approximately 1000. These results were helpful for the establishment of the following purification method for the beet antagonist.

Purification of the Bradykinin Antagonist from the Beet

Step 1——Two kilograms of grated beet roots were lyophilized, and usually 300—350 g of the dried material was obtained. It was transferred to a flask of 5 liters capacity, and 4 liters of 99% (v/v) ethyl alcohol were added. A cooler was attached at the top of the flask. The material was then heated in a boiling water bath for 6 hr. After cooling, the residue was filtered off and was extracted further three times. The combined filtrate was evaporated to dryness *in vacuo* in a rotary evaporator. The residue obtained was dissolved in 500 ml

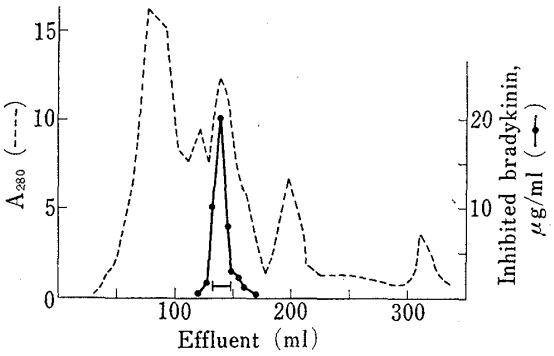

Fig. 3. Gel Filtration of the Sample HI on a Sephadex G-15 Column

sample, 5 ml of the sample HI; column size, 1.5×80 cm; solvent, 0.15M NaCl (pH 6.8)
The bracketed part of the effluent was combined.

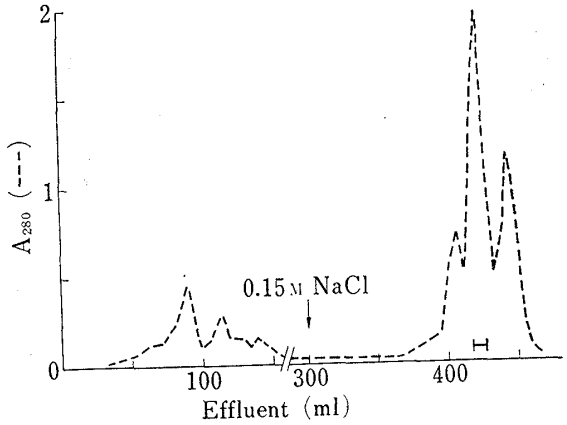

Fig. 4. Gel Filtration of the Sample G on a Bio-Gel P-2 Column

sample, 2 ml of the sample G; column size, 1.5×50 cm
The elution was conducted first with distilled water, and then with 0.15M NaCl (pH 6.8). The bracketed part was combined

11) Y. Hojima, M. Tanaka, H. Moriya, and C. Moriwaki, *Arerugi*, **20**, 763 (1971); K. Kizuki, Y. Hojima, H. Moriya, C. Moriwaki, and T. Nakajima, The 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, Apr., 1972.
12) H. Wyler and A.S. Dreiding, *Helv. Chim. Acta*, **40**, 191 (1957); M. Piattelli and L. Minale, *Phytochem.*, **3**, 307 (1964); *idem*, *ibid.*, **3**, 547 (1964).
13) G.W. Pucher, L.C. Curtiris, and H.B. Vickery, *J. Biol. Chem.*, **123**, 61 (1938).
14) H. Wyler and A.S. Dreiding, *Helv. Chim. Acta*, **42**, 1699 (1959); *idem*, *ibid.*, **45**, 638 (1962).

of water and then shaken with the same volume of ether for 5 min. The ether soluble materials were mostly removed by repeating this treatment three times. The water layer pooled was concentrated to a small volume *in vacuo* in a rotary evaporator and the final volume was adjusted with 0.15M NaCl to 100 ml (sample HI). The recovery of the activity at this stage was 45—65%.

Step 2——A part of the sample HI, equivalent to 100 g beet roots, was applied to a Sephadex G-15 column and eluted with 0.15M NaCl (pH 6.8). The optical density of each fraction was measured at 280 nm, and assayed for its inhibitory potency to the bradykinin effect upon the contraction of isolated guinea-pig ileum (Fig. 3). The active fractions were pooled (sample G). The recovery of the activity was about 30—50% against that of the beet extract, and the antagonist was purified about 15-fold.

Step 3——The sample G obtained from step 2 was evaporated to dryness *in vacuo* by a rotary evaporator. The active substance was extracted with anhydrous methyl alcohol and the insoluble materials were removed by centrifugation, by which the majority of NaCl contained in the sample was able to be removed. Methyl alcohol was then evaporated *in vacuo*, and the residue, after dissolved in 2 ml of distilled water, was applied to a Bio-Gel P-2 column. The elution was conducted first with distilled water and then with 0.15M NaCl (pH 6.8). The active fraction (sample P) was eluted with the second eluant 0.15M NaCl (Fig. 4). The recovery of the activity was 10—15%, and the antagonist was purified 25-fold through the whole purification. Its specific activity, μg of inhibited bradykinin per A_{280}, was approximately 1.5.

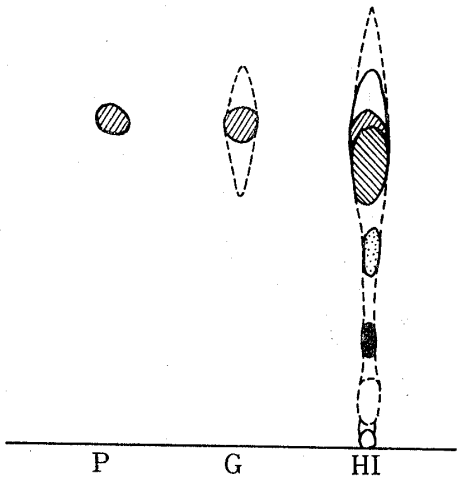

Fig. 5. Thin-Layer Chromatogram of the Samples P, G and HI

layer, Silica Gel H, 0.3 mm in thickness; solvent, *n*-butanol: acetic acid: water (4: 1: 5); detection, iodine vapour; developing time, 40 min

The sample P was detected with iodine vapour as a single spot on a silica gel thin-layer chromatogram developed with the solvent system of *n*-butanol: acetic acid: water (4: 1: 5) (Fig. 5).

The ultraviolet absorption spectrum of the sample P was measured in 0.15M NaCl (pH 6.8). The maximum absorption was observed at 282 nm (Fig. 6).

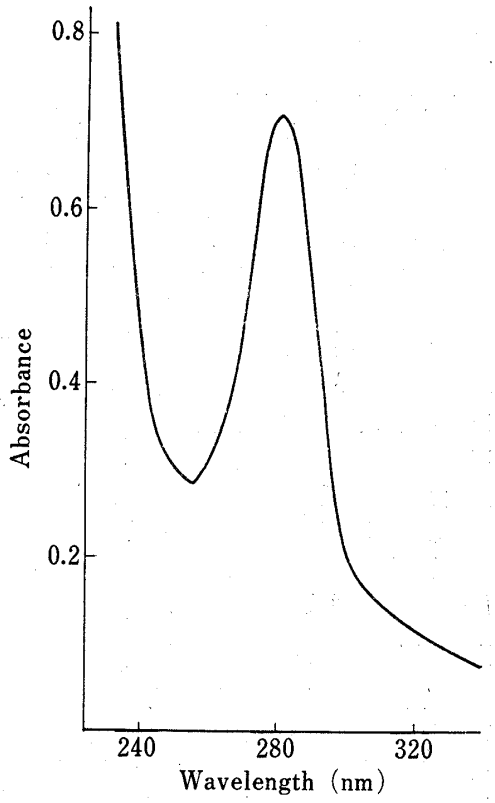

Fig. 6. Ultraviolet Absorption Spectrum of the Sample P in 0.15M NaCl (pH 6.8)

The sample P was diluted 2 times with 0.15M NaCl (pH 6.8).

Inhibitory Effect to Bradykinin, Histamine and 5-HT

The sample HI was diluted with saline 10 times (HI×10), 50 times (HI×50) and 100 times (HI×100). Each concentration of diluted sample HI was examined for its inhibitory effect on the contractions of the isolated guinea-pig ileum induced by bradykinin, histamine, and 5-HT.

Figure 7 shows the inhibitory effect of the sample HI on the bradykinin effect upon the guinea-pig ileum. The inhibitory effect was easily removed from the tissue preparation by washing, thereafter not leaving any change of the sensitivity of the tissue to bradykinin (A of Fig. 7). Moreover it was characteristic that the contraction induced by 80 ng of bradykinin rapidly disappeared after the infusion of HI×10 (B of Fig. 7). This type of antagonistic effect against bradykinin was found in all inhibitory fractions of the present investigation. Such effects against histamine and 5-HT but not against acetylcholine were also detected in some samples, but those were weak.

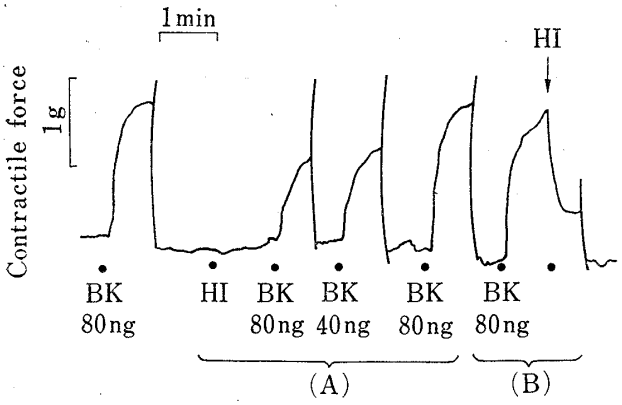

Fig. 7. Antagonistic Effect of the Sample HI on the Contraction of Isolated Guinea-Pig Ileum

(A): 0.1 ml of the 10 times diluted sample HI (HI×10) was infused into the organ bath 1 min before the addition of 80 ng of bradykinin (BK).
(B): 0.1 ml of HI×10 was infused into the bath when the maximal contraction by 80 ng bradykinin was induced.

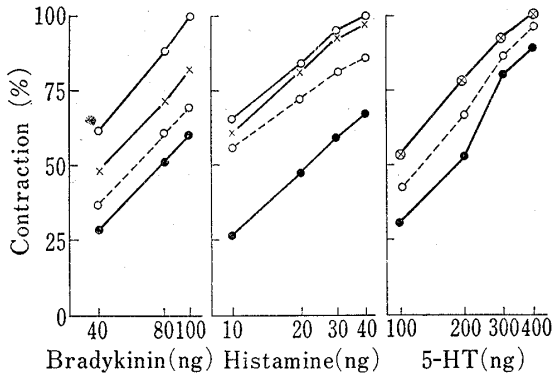

Fig. 8. Inhibitory Effect of the Sample HI on the Contractions of the Guinea-Pig Ileums Induced by Bradykinin, Histamine and 5-HT

Each 0.1 ml of the diluted samples (10, 50 and 100 times) was infused into the organ bath, and 1 min later respective doses of each agonist (0.2 ml) were injected. —○—, control; —●—, HI×10; --○--, HI ×50; —×—, HI×100

The sample HI was also inhibitory to histamine and 5-HT (Fig. 8). The inhibitory effect of each concentration of the sample on bradykinin, histamine and 5-HT was compared in Fig. 9. Both inhibitory percentages of HI×10 against 100 ng bradykinin and 40 ng histamine were about 70%, and that against 400 ng of 5-HT was about 30%. The sample HI×100 had no effect on 5-HT, while it was effective on the actions of bradykinin and histamine with about 20% and 10% inhibitions, respectively. As for the inhibitory profile on bradykinin, the linear relationship was usually observed between the sample dosage in logarithm and the inhibitory percentage, as shown in Fig. 9.

Inhibition of Vasodilatation

The 10 times diluted sample G in a dose of 0.1 ml was injected together with 20 ng of bradykinin (0.1 ml) into the femoral artery of a dog. The sample G obviously reduced the vasodilating activity of bradykinin as shown in Fig. 10, and that was independent of the incubation time with bradykinin.

Inhibition of the Increase of Capillary Permeability

Rats were injected with pontamine sky blue 6B, and 5 min later were intradermally injected with 0.01, 0.1, 1 and 10 μg of bradykinin or 0.1, 1, 10 and 100 μg of histamine in a

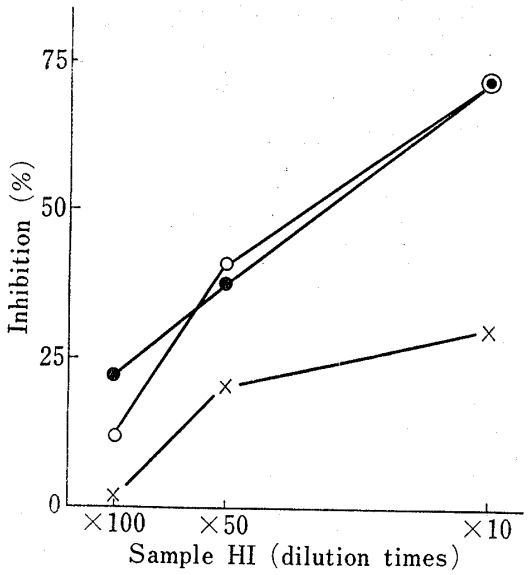

Fig. 9. Relationship between the Amounts of the Antagonist Sample HI and Three Contracting Agents

For the experimental conditions, see the legend of Fig. 8. —●—, bradykinin (100ng); —○—, histamine (40 ng); —×—, 5-HT (400 ng)

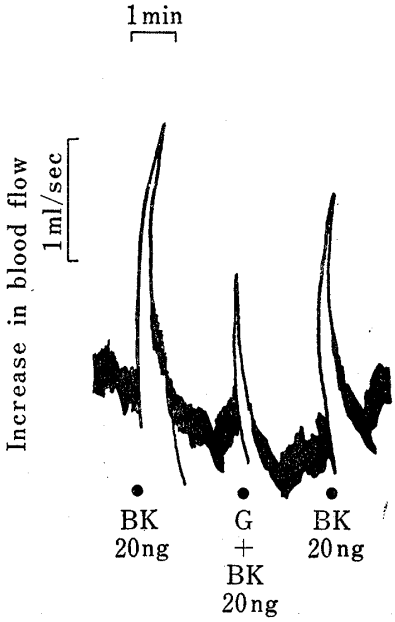

Fig. 10. Inhibitory Effect of the Sample G on the Vasodilating Activity of Bradykinin

0.1 ml of the 10 times diluted of the sample G or saline for the control was mixed with 20 ng bradykinin (BK) (0.1 ml) and injected into the dog femoral artery.

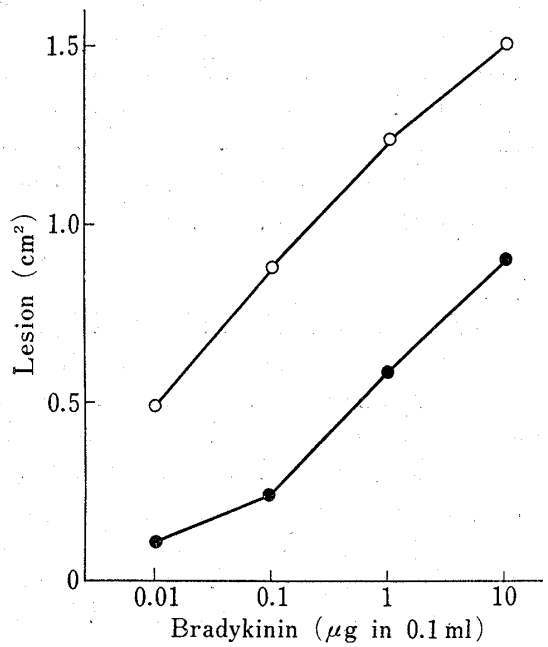

Fig. 11. Inhibition of the Increase of Capillary Permeability Induced by Bradykinin

—○—, control; —●—, sample G (1 ml/kg), administered intravenously 5 min before the intradermal injection of bradykinin

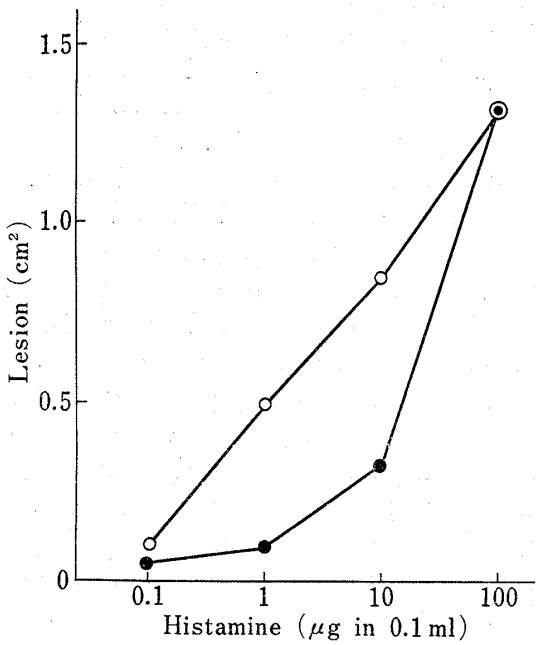

Fig. 12. Inhibition of the Increase of Capillary Permeability Induced by Histamine

—○—, control; —●—, sample G (1 ml/kg), administered intravenously 5 min before the intradermal injection of histamine

volume of 0.1 ml. The dosal response curve for each agonist was obtained based on the areas of the blue spots. The sample G in a dose of 1 ml/kg was intravenously administered, and 5 min later intradermal injections of the agonists were repeated. The inhibitory effect on

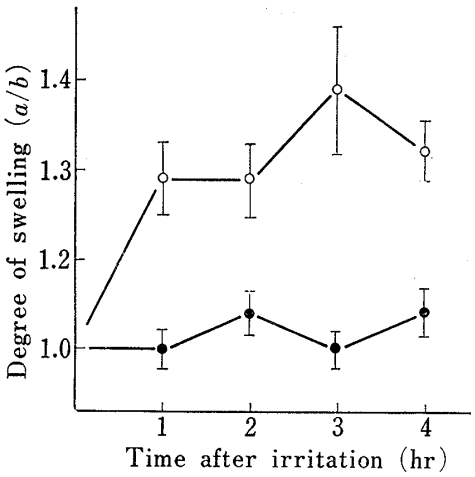

Fig. 13. Effect of the Sample G on Carrageenin-Induced Edema in the Rat Hind Paw

a, volume of the hind paw after carrageenin treatment; *b*, volume of the hind paw before treatment
—○—, control, saline (5 ml/kg) injected intraperitoneally; —●—, sample G (5 ml/kg)

the increase in the capillary permeability induced by bradykinin and histamine was clearly observed (Fig. 11 and 12). However the blueing by 100 μg of histamine was not suppressed.

Inhibition of Rat Paw Edema

Figure 13 shows the inhibitory effect of the sample G on the carrageenin-induced rat paw edema. The sample was administered intraperitoneally with a dose of 5 ml/kg.

Discussion

Thus the new, naturally occurring antagonist of bradykinin was discovered in the beet roots. The content of this antagonist in the beet root did not extensively vary according to harvest periods. In most cases, larger roots (about 150—250 g per root) were used for the present experiments.

For the preparation of a crude sample like the sample HI, the following useful method has been also developed. The grated fresh beet roots were boiled in 95% ethyl alcohol. After evaporation of the alcohol, the residue was dissolved in water and then treated with ether. Lead acetate solution was added until about 2.5% (w/v) to the aqueous layer separated from the ether layer, and the precipitate formed was discarded by centrifugation. The majority of the red-violet pigment was able to be removed in this manner. Hydrogen sulfide was gassed into the supernatant to remove the excess lead. Then the obtained clear filtrate was adjusted to pH 6—7 by adding $CaCO_3$, and insoluble $CaCO_3$ was filtered off. The filtrate was concentrated to a small volume *in vacuo* in a rotary evaporator, and a syrupy liquid was obtained. It was transferred to a flat beaker and stored overnight in a cold room. Much quantities of needle crystals were produced during the storage and filtered off by suction. The recovery of the activity of the filtrate was 40—60% through the procedures, and the filtrate was a useful sample for the further purification by gel chromatographies like steps 2 and 3.

The partially purified antagonist from the beet was effective on both *in vitro* and *in vivo* experiments such as the contraction of the isolated guinea-pig ileum, the vasodilator activity in dog and the capillary permeability increase in rat, which induced by bradykinin (Fig. 7—11). Although the antagonistic mechanism of this substance cannot be explained at present, it is assumed to react with bradykinin receptors. Rocha e Silva distinguished two kinds of bradykinin antagonists in terms of the receptor concept[15]: (1) those acting upon receptors in the guinea-pig bronchioli, in a certain way correlated to pain receptors, and (2) those acting upon the guinea-pig ileum which might be correlated to receptors in the venular side of the vascular bed responsible for edema formation. The results obtained by the present *in vitro* and *in vivo* experiments show that the antagonist purified from the beet satisfies the latter category.

All of the bradykinin antagonists reported to date,[2-6] are synthetic compounds. Most of them have not always satisfactory effect, and they are occasionally accompanied with potentiating effect on bradykinin activity depending on their concentrations. However, with the antagonist that the authors purified from the beet, the potency of anti-bradykinin activity increased in relation to the use of higher concentration. The linear relation between

15) M. Rocha e Silva, "Kinin Hormones," Charles C. Thomas Publisher, Springfield, Illinois, 1970, p. 218.

the inhibitory percentage and the logarithm of this antagonist concentration was observed (Fig. 9). The antagonist by itself produced no response on the isolated guinea-pig ileum even in higher concentration, but suppressed the contractile response induced by bradykinin and produced a marked fall in tonus when acted on the contracted ileum by bradykinin (B of Fig. 7).

Furthermore, this substance showed the strong anti-inflammatory activity on the carrageenin-induced edema in rats. Although the anti-inflammatory mechanism of this substance could not be easily analyzed since the inflammation process is quite complicated, it may be considered to antagonize against the liberated kinins by the activation of kallikrein-kinin system through the activation of Hageman factor by carrageenin.[16] On the other hand, Vargaftig and Dao Hai[17] implied that bradykinin evoked the release of "rabbit aorta contracting substance (RCS)" relating to the inflamed tissue. The possibility of the beet antagonist blocking this process like non-steroidal anti-inflammatory agents[18] could be also speculated.

On the anaphylaxis of rat, West and his coworkers proposed two phases on shock mediators[19]: an early phase, 10 days after sensitization with horse serum in which bradykinin is considered to be a main mediator, and a late phase, 20 days after sensitization when bradykinin is not involved. To test the anti-anaphylactic shock, 2 ml/kg or 0.5 ml/kg of the sample G was intravenously administered into rats sensitized with horse serum, and then the rats were challenged with the antigen as previously reported[20]; however, the beet antagonist sample G was not effective on the inhibition of anaphylactic mortality at either the early or the late phase.

Studies on the further detailed properties including the chemical identity of the beet antagonist, are now under hard investigation.

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