[Chem. Pharm. Bull. 29(11)3369—3374(1981)]

Potentiating Effects of Bradykinin-related Substances on Bradykinin-induced Contraction of Guinea Pig Ileum and Hypotension in Rats

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(Received June 24, 1981)

Bradykinin-related substances were studied *in vitro* and *in vivo* for bradykinin-potentiating activity. Of the ten peptides which augmented the contractile response of isolated guinea pig ileum to bradykinin, des-Pro^2-bradykinin was the most potent. Its potency was 1.9 times that of potentiator B, a bradykinin potentiating peptide obtained from the venom of *Agkistrodon halys blomhoffii*. This peptide also potentiated the vasodepressing activity of bradykinin in rats and inhibited angiotensin converting enzyme. These findings indicate that des-Pro^2-bradykinin, like venom peptides, potentiates the activity of bradykinin, possibly by inhibiting angiotensin converting enzyme (kininase II).

Keywords——bradykinin-related substances; potentiation; contraction of guinea pig ileum; blood pressure in rat; angiotension converting enzyme; SQ 14225

Some venom peptides are known to potentiate bradykinin activity. In 1965, Ferreira[1] reported that a mixture of peptides obtained from the venom of *Bothrops jararaca* potentiated bradykinin activity.

Kato and Suzuki[2a-c] isolated five different undecapeptides (including potentiator B) which enhanced bradykinin activity from the venom of Japanese pit viper *Agkistrodon halys blomhoffii*. They reported that some of these peptides inhibited the activity of angiotensin converting enzyme.[3a-d]

We synthesized several bradykinin-related peptides and studied them for bradykinin-potentiating activity on isolated guinea pig ileum and blood pressure in rats, using potentiator B and SQ 14225, respectively, as the control substances, the latter being an angiotensin converting enzyme inhibitor. Des-Pro^2-bradykinin was examined for inhibitory effect on angiotensin converting enzyme.

Materials and Methods

Bradykinin-related peptides used were synthesized by elongation from C-terminal arginine by a method described in our previous papers.[4a-d] *p*-Methoxybenzyloxycarbonyl and *tert*-amyloxycarbonyl were applied for the protection of alpha-amino groups and a nitro group was used for protection of the ω-guanidino moiety of the arginine residue.

The final fully protected peptides were deprotected by the HF/anisole procedure, and then purified by recrystallization or ion-exchange chromatography on carboxymethyl-cellulose followed by column chromatography on Sephadex LH-20.

The structures, physical constants and analytical data of synthetic peptides are listed in Table I.

Augmentation of Bradykinin-induced Contraction of Guinea Pig Ileum by Bradykinin-related Substances——The ileum, isolated from male Hartley strain guinea pigs weighing 300 to 400 g, was suspended in 10 ml of well-aerated Tyrode solution kept at 36—38°C. The activity was measured in terms of concentration of test substances doubling the effect of a single dose of bradykinin. Each experiment was repeated four times. Test substances were added 1 min before addition of bradykinin and their effects were compared with that of potentiator B, a bradykinin-potentiating peptide obtained from the venom of *Agkistrodon halys blomhoffii*.

TABLE I. Structures, Physical Constants and Analytical Data of Bradykinin-related Substances

Peptides	Formula	$[\alpha]_D^{25}$	(c, Solvent)	Elemental analysis Calcd (Found) C	H	N	Amino acid analysis Calcd (Found) Arg	Pro	Gly	Ser	Phe
I[4d]	$C_{49}H_{84}N_{14}O_{19}$	−58.4	(0.5 1%AcOH)	50.15	7.22	16.17	2	2	1	1	2
				(50.31	7.53	17.08)	(2.00	1.89	1.00	0.89	2.02)
II	$C_{41}H_{66}N_{10}O_{15}$	−50.4	(0.4 H_2O)	52.44	7.08	14.92)	1	2	1	1	1
				(52.36	6.53	15.16)	(1.01	1.82	0.92	0.74	2.00)
III	$C_{46}H_{81}N_{13}O_{20}$	−50.7	(0.5 H_2O)	48.63	7.19	16.03	2	1	1	1	2
				(48.04	6.65	15.91)	(1.97	1.14	0.81	1.00	2.41)
IV	$C_{42}H_{78}N_{12}O_{19}$	−34.6	(0.4 H_2O)	47.81	7.45	15.93	2	1		1	2
				(48.07	7.16	16.19)	(1.86	1.01		0.84	2.00)
V	$C_{34}H_{52}N_8O_{11}$	−37.0	(0.5 H_2O)	54.53	7.00	14.96	1	1		1	2
				(54.51	7.07	15.15)	(1.02	1.05		0.89	2.00)
VI	$C_{33}H_{59}N_{11}O_{13}$	−40.4	(0.6 H_2O)	48.46	7.27	18.84	2	1		1	1
				(48.26	7.61	18.40)	(2.00	1.02		0.80	1.00)
VII	$C_{31}H_{58}N_{10}O_{13}$	−33.0	(0.7 H_2O)	47.80	7.74	17.94	2	1			1
				(47.28	7.60	18.21)	(2.03	1.06			1.00)
VIII	$C_{23}H_{36}N_6O_7$	−21.6	(0.5 H_2O)	54.32	7.14	16.53	1	1			1
				(54.35	7.51	16.52)	(1.00	0.97			1.04)
IX	$C_{27}H_{48}N_9O_{10.5}$	+ 7.7	(0.6 DMF)	48.64	7.26	18.91	2	1			
				(49.00	7.46	18.83)	(2.00	0.97			)
X	$C_{16}H_{36}N_8O_8$	+15.1	(0.7 H_2O)	41.02	7.75	23.92					
				(40.87	8.01	24.13)					

I Arg–Pro–Gly–Phe–Ser–Pro–Phe–Arg 2AcOH $5H_2O$; II Pro–Gly–Phe–Ser–Pro–Phe–Arg AcOH $4H_2O$; III Arg–Gly–Phe–Ser–Pro–Phe–Arg 3AcOH $5H_2O$; IV Arg–Phe–Ser–Pro–Phe–Arg 2AcOH $7H_2O$; V Phe–Ser–Pro–Phe–Arg 0.5AcOH $3H_2O$; VI Arg–Ser–Pro–Phe–Arg 2AcOH $2H_2O$; VII Arg–Pro–Phe–Arg 2.5AcOH $3H_2O$; VIII Pro–Phe–Arg 1.5AcOH; IX Arg–Phe–Arg 3AcOH $0.5H_2O$; X Arg–Arg 2AcOH H_2O.

Potentiation of Bradykinin Vasodepressing Responses in Anesthetized Rats by Des-Pro²-bradykinin——Groups of six to eight Wistar male rats weighing 350 to 550 g were anesthetized with 1 g/kg of urethane *i.p.* and the trachea was cannulated with a polyethylene tube. Blood pressure, measured from the carotid artery with a pressure transducer (Nihon Koden MPUOS 290), was recorded on a polygraph recorder (Nihon Koden RM-85).

Des-Pro²-bradykinin, which proved to be the most potent of all the bradykinin-related substances tested on guinea pig ileum, was compared with SQ 14225. Drugs were injected into the femoral vein.

Inhibition of Angiotensin Converting Enzyme by Des-Pro²-bradykinin——The enzyme activity was assayed by the procedure described by Cushman.[5] The powder which was precipitated by addition of acetone to extract of rabbit lung was suspended in 50 mM phosphate buffer (pH 8.3). The suspension was centrifuged for 40 min at 24000 rpm, and the supernatant was used as the angiotensin converting enzyme preparation. The enzymatic activity was assayed spectrophotometrically (at 228 nm) in terms of the rate of formation of hippuric acid from Hip–His–Leu, a substrate. The enzymatic reaction was performed by addition of 50 μl of the enzyme and incubation at 37°C for 30 min, and terminated by addition of 0.25 ml of 1 N HCl. The IC_{50} values, or the concentration required for inhibition of angiotensin conversion by 50%, were determined.

Results

Augmentation of Bradykinin-induced Contraction in Guinea Pig Ileum by Bradykinin-related Substances

Ten bradykinin-related peptides and arginine were compared with potentiator B, a bradykinin-potentiating peptide obtained from the venom of *Agkistrodon halys blomhoffii*. The results are shown in Table II and in Fig. 1.

When pretreatment with des-Pro²-bradykinin (5×10^{-7} M) preceded the addition of a single dose of bradykinin, the ileum contraction was higher than that induced by a doubled bradykinin dose.

Table II. Potentiation of Bradykinin-induced Contraction of Guinea Pig Ileum by Bradykinin-related Substances

Peptides	Concentration[a] $\times 10^{-6}$ M
I	0.20±0.09
II	5.5 ±0.11
III	0.82±0.01
IV	2.7 ±0.16
V	59.0 ±1.35
VI	6.0 ±0.09
VII	6.7 ±0.14
VIII	123.0 ±0.77
IX	42.0 ±0.35
X	>500.0
Arg	340.0±9.35
XI	0.38±0.11

a) Concentration of peptides in tyrode solution required to double the bradykinin activity. XI: Pyr-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro (Potentiator B)
Each value is a mean of four experiments.

Nine bradykinin-related peptides potentiated the activity of bradykinin at concentrations of 2×10^{-7} M to 3.4×10^{-4} M. Arg-Arg, however, failed to augment the contraction at 5×10^{-4} M. Des-Pro^2-bradykinin was the most potent, with an activity 1.9 times that of potentiator B, but it was devoid of any potentiating effect on acetylcholine-induced contraction.

Bradykinin-related substances were without appreciable effect on resting tonus or intrinsic ileum activity at concentrations augmenting the bradykinin activity.

Potentiation of Vasodepressing Responses to Bradykinin in Anesthetized Rats by Des-Pro^2-bradykinin

The dose-response relationship of bradykinin on blood pressure in anesthetized rats is shown in Fig. 2. Vasodepressing responses to bradykinin at *i.v.* doses of 0.1, 1, 3 and 10 μg/kg were −4.9±0.7, −8.3±1.2, −15.8±1.2 and −26.3±1.8 mmHg (M.E.+S.E.), respectively. At single intravenous doses, des-Pro^2-bradykinin (0.1—1 mg/kg) slightly elevated blood pressure (by 2—15 mmHg), which returned to the basal level within 5 min, but SQ 14225 (0.03 mg/kg), in contrast, slightly lowered blood pressure (by 5—15 mmHg) though again it returned to the basal level within 5 min (Fig. 3).

The effect of intravenously given doses of test substances on blood pressure was as follows. Des-Pro^2-bradykinin, at 0.03 mg/kg, produced no marked change in response to bradykinin,

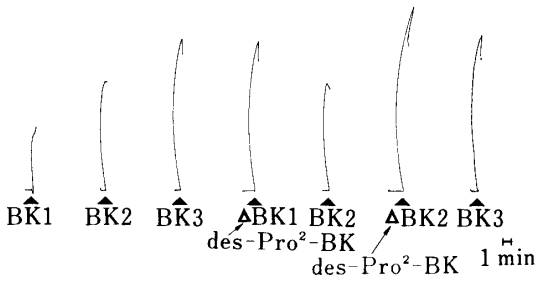

Fig. 1. Effects of Des-Pro^2-Bradykinin on Contractile Responses of Isolated Guinea pig Ileum to Bradykinin

Bradykinin was added at B1, B2 and B3 at doses of 2×10^{-8} M, 4×10^{-8} M, 8×10^{8} M, respectively. Des-Pro^2-bradykinin (Des, 5×10^{-7} M) was applied 1 min before bradykinin.

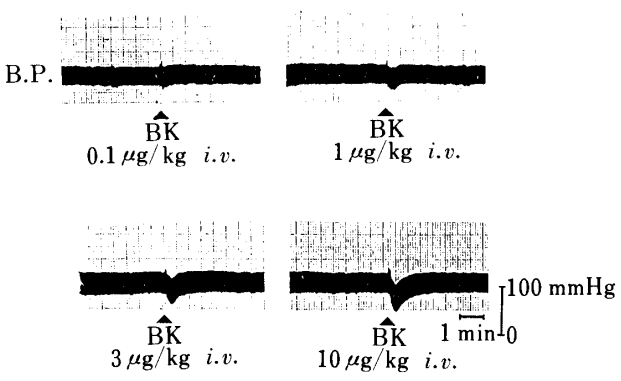

Fig. 2. Effects of Bradykinin on Carotid Arterial Blood Pressure of Anesthetized Rats

Bradykinin was injected *i.v.* at doses of 0.1, 1, 3 and 10 μg/kg.

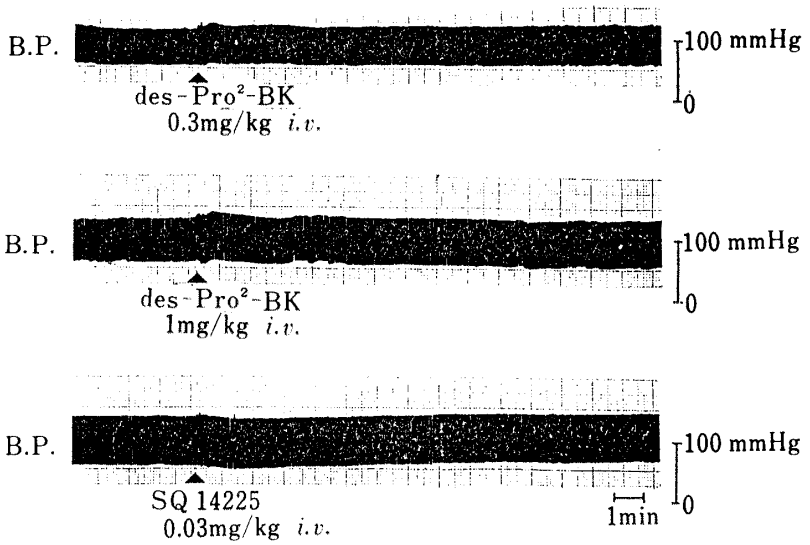

Fig. 3. Effects of Des-Pro²-Bradykinin and SQ 14225 on Carotid Arterial Blood Pressure of Anesthetized Rats

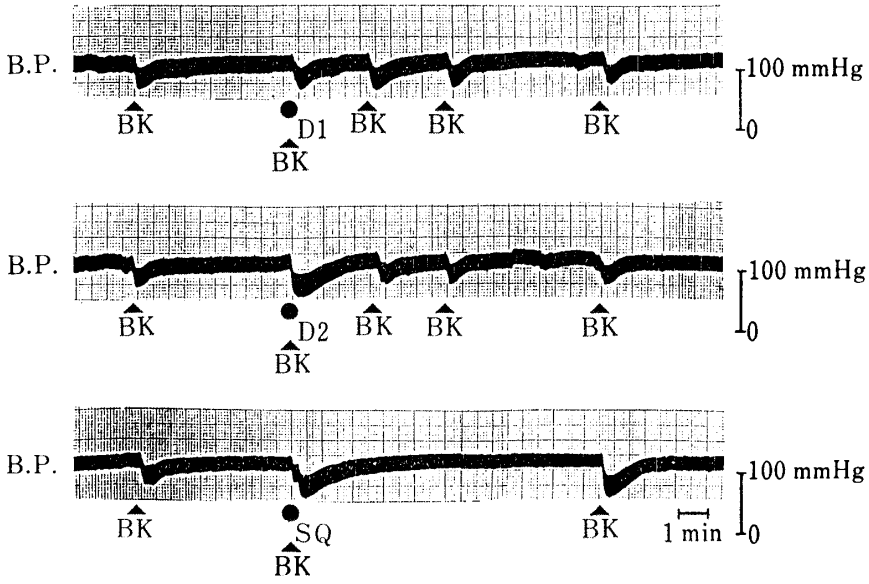

Fig. 4. Effects of Des-Pro²-Bradykinin and SQ 14225 on Bradykinin Vasodepressive Responses of Anesthetized Rats

Des-Pro²-bradykinin was injected *i.v.* at D1 (0.1 mg/kg) and D2 (0.3 mg/kg). SQ 14225 was injected *.v.* at SQ (0.03 mg /kg). Bradykinin was injected at BK (before, simultaneously with, and 2.5, 5 and 10 min after treatment with des-Pro²-bradykinin or SQ 14225).

but at a larger dose of 0.1 mg/kg, the response was augmented, with recovery occurring within the next 2.5 min. SQ 14225 augmented the response to bradykinin administered concurrently with or after SQ 14225 (Fig. 4, Fig. 5). The vasodepressing response to acetylcholine, however, did not change appreciably after treatment with des-Pro²-bradykinin.

Inhibition of Angiotensin Converting Enzyme by Des-Pro²-bradykinin

In order to elucidate the mechanism of this inhibition, we tested the inhibitory activity of des-Pro²-bradykinin. Figure 6 shows the dose-dependent inhibitory response. The concentration required for 50% inhibition (IC_{50}) was 2.7 μM.

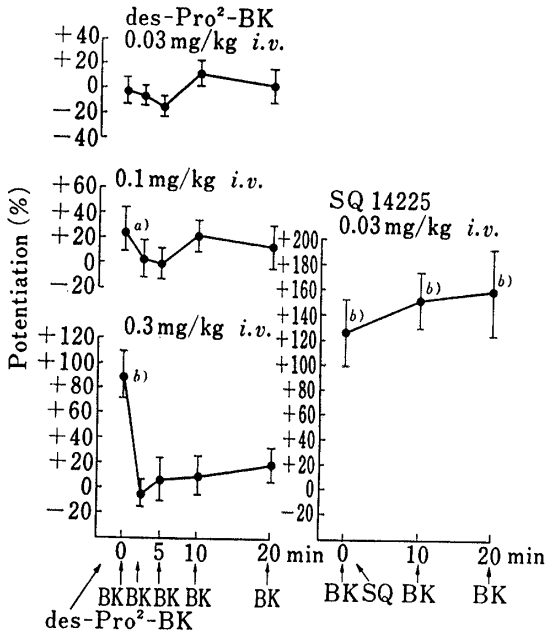

Fig. 5. Changes in *i.v.* Bradykinin-induced Vasodepression by Des-Pro²-Bradykinin and SQ 14225

In this experiment, groups of six to eight animals were used. Des-Pro²-bradykinin (0.03, 0.1 or 0.3 mg/kg) or SQ 14225 (0.03 mg/kg) was injected *i.v.* at 0 min, and bradykinin (3 ug/kg) was injected at 0, 2.5, 5, 10 and 20 min or at 0, 10 and 20 min. Changes are expressed as percentages of the basal level. The vertical bars represent S.E.M

a) $p<0.05$ as compared with bradykinin alone,
b) $p<0.01$ as compared with bradykinin alone.
The paired *t*-test was used.

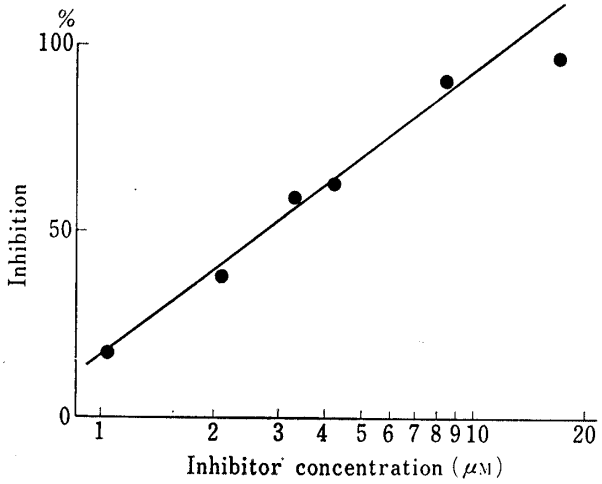

Fig. 6. Relationship of Des-Pro²-Bradykinin Concentration to Inhibition of Angiotensin Converting Enzyme

Discussion

Bradykinin-related substances *per se* did not contract guinea pig ileum at concentrations high enough to augment bradykinin activity. Unlike bradykinin, des-Pro²-bradykinin did not lower the blood pressure. These findings suggest the absence of bradykinin-like activity in bradykinin-related substances at those concentrations. As shown in Table II, bradykinin-related substances become more potent as their structure becomes closer to that of bradykinin. It is also noteworthy that an arginine residue attached to the N-terminal of a peptide potentiated the activity by 18.4 to 27.5 times. Bradykinin-related substances differ from venom peptides in that they are devoid of N-terminal pyrrolidone carboxylic acid, which is a characteristic of most venom peptides.[3b,c] Peptides from the venom of *Bothrops jararaca*[1,3a,6] or *Agkistrodon halys blomhoffii*,[2c] as well as SQ 14225[7] which are known to potentiate bradykinin activity, inhibit an enzyme which converts angiotensin I to angiotensin II and inactivates bradykinin.[3a,b] It is supposed that the bradykinin-potentiating activity of des-Pro²-bradykinin is due in part to the inhibition of the angiotensin converting enzyme, which was found to be identical to kininase II[6] or bradykinin degrading enzyme.[8]

Des-Pro²-bradykinin inhibited the angiotensin converting enzyme. Therefore, we speculated that the bradykinin-potentiating activity of des-Pro²-bradykinin might be due in part to inhibition of this enzyme. Most venom peptides and bradykinin itself have been found to act as competitive inhibitors of angiotensin converting enzyme.[3a,5,9a,b] Des-Pro²-bradykinin, which is structurally similar to bradykinin, might therefore also inhibit the enzyme competitively. SQ 20475, which is a pentapeptide (Pyr–Lys–Trp–Ala–Pro) from

Bothrops jararaca lacking a penultimate proline residue, is degraded by angiotensin converting enzyme. This degradation may contribute to the short duration of inhibition by this pentapeptide *in vitro* and *in vivo*.[3a] Des-Pro^2-bradykinin also showed shortterm bradykinin-potentiating activity. Des-Pro^2-bradykinin, being devoid of a proline residue in the second position from the C-terminal, may be degraded by angiotensin converting enzyme.

During the preparation of this report, it was shown that some bradykinin fragments, Arg–Pro, Arg–Pro–Pro, Phe–Ser–Pro, inhibit angiotensin converting enzyme of rat brain.[10]

Acknowledgement We thank Dr. Okaji of Tokyo Research Laboratory of Kyowa Hakko Kogyo Co., Ltd. for his help in measuring angiotensin converting enzyme activity.

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