CONTRACTILE RESPONSES OF ISOLATED DOG MESENTERIC ARTERIES TO ANGIOTENSIN I, II AND III

Noboru TODA, Shigehiro HAYASHI and Mizuo MIYAZAKI

Department of Pharmacology, Shiga University of Medical Sciences.
Seta, Ohtsu 520-21, Japan

Accepted January 22, 1978

Abstract—The addition of angiotensin (Ang-) I, II and III caused a dose-dependent contraction of helically cut strips of dog mesenteric arteries. Tachyphylaxis developed following repeated additions of angiotensins. Average median effective concentrations of Ang-I, II and III were 3.7, 0.8 and $2.5 \times 10^{-8}$ M, respectively. Contractile responses to the angiotensins were attenuated to a similar extent by Ang-II antagonists, Sar1 Ileu8 Ang-II and Sar1 Ala8 Ang-II, but were unaffected by phentolamine, methysergide and diphenhydramine. The response to Ang-I was significantly reduced by treatment with bradykinin-potentiator B, while the response to Ang-II was not influenced. It may be concluded that Ang-I, II and III produce contractions possibly by activation of same Ang-II receptors and that contractions induced by Ang-I are associated, to some extent, with a conversion to Ang-II in the arterial wall.

Angiotensin II (Ang-II), a potent vasoconstrictor octapeptide, is formed in vivo from angiotensinogen by the catalysis of renin and angiotensin I (Ang-I) converting enzyme. Ang-I, a decapeptide, produces vasoconstriction, possibly via a conversion to Ang-II mainly in the lung in vivo (1). Such conversion has been suggested to occur also in the isolated vascular wall (2). The vasoconstricting effect of angiotensin III (Ang-III), a heptapeptide, is markedly less than that of Ang-II in rabbit aortic strips, while a greater secretion of aldosterone from rabbit adrenal cortex is induced by Ang-III (3).

The present study was undertaken to compare responses of isolated dog mesenteric arteries to Ang-I, II and III and to investigate the mechanism of action of Ang-I and III.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 7 to 16 kg, were anesthetized with 50 mg/kg sodium pentobarbital given i.p. sacrificed by bleeding from the common carotid arteries. The distal portion of the superior mesenteric arteries (0.6 to 0.9 mm outside diameter) was isolated and the arteries were helically cut into strips, approx. 20 mm long. The specimens were vertically fixed between hooks under an optimal resting tension of 1.5 g in a muscle bath containing the nutrient solution which was maintained at 37±0.5°C and aerated with mixture of 95% O2 and 5% CO2. Hooks anchoring the upper end of the strips were connected to the lever of a force-displacement transducer (Sanei Sokki Co., Tokyo). Constituents of the solution were as follows (mM): Na+, 162.1; K+, 5.4; Ca++, 2.2; Mg++, 1.0, Cl−, 159.0; HCO3−, 14.9; and dextrose, 5.6. The pH of the solution was 7.2 to 7.3. Before the start of experiments, preparations were allowed to equilibrate for 90 to 120 min.
in control media, during which time the bathing fluids were replaced every 15 to 20 min. The contractile response to 30 mM K⁺ was first obtained, and responses to angiotensins relative to contractions induced by 30 mM K⁺ are presented. Contractions were displayed on an ink-writing oscillograph (Sanei Sokki Co.). After the response to K⁺ or angiotensins given in a single dose was completed, the preparations were washed three times with fresh fluids and equilibrated for 30 to 40 min. Preparations were treated for 20 min with blocking agents before the addition of angiotensins. Drugs used were Ang-I (Ileu⁵-Ang-I, Protein Research Foundation, Osaka; P. R. F.), Ang-II (Ileu⁵-Ang-II, P. R. F.), Ang-III (des-Asp⁴ Ang-II, P. R. F.), bradykinin-potentiator B (P. R. F.), Sar¹ Ileu⁸ Ang-II (P. R. F.), Sar¹ Ala⁸ Ang-II (P. R. F.), phentolamine mesylate, methysergide bimaleate and diphenhydramine hydrochloride.

The results shown in the text, figures and tables represent mean values ± standard errors of the means. Statistical analyses were made using the Student's t-test.

RESULTS

Comparison of responses to Ang-I, II and III

In mesenteric arterial strips, maximum contractions were elicited in response to 5 × 10⁻⁷ M Ang-I, 10⁻⁷ M Ang-II and 5 × 10⁻⁷ M Ang-III. Angiotensins in these concentrations caused a transient contraction, and when the tension returned to the level prior to the addition of the drugs, additional application of angiotensins (10⁻⁷ to 10⁻⁵ M) failed to significantly alter the tension. Therefore, the response to angiotensins only in a single concentration was obtained, and preparations were repeatedly washed. Contractions induced by Ang-I and II were always greater at the first trial of application than at the second (50 to 60% the contraction of first trial); however, after the second trial, the responses were approx. the same (Fig. 1) when the angiotensin-added fluids were replaced three times with control media and preparations were equilibrated for 30 to 40 min. Similar results were obtained with Ang-III. Therefore, further studies on dose-response relationships and with blocking agents were carried out in preparations in which the response to angiotensins was stabilized. Cross tachyphylaxis of the responses to Ang-I, II and III occurred.

The addition of Ang-I (5 × 10⁻⁹ to 5 × 10⁻⁷ M), Ang-II (10⁻⁸ to 10⁻⁷ M) and Ang-III (5 × 10⁻⁹ to 5 × 10⁻⁷ M) caused a dose-related contraction of mesenteric arterial strips; further increase in the concentrations produced less contractions (Fig. 2). The pattern of contractions induced at various concentrations of angiotensins was quite different: at low concentrations, a slight, persistent contraction and at high concentrations, a marked, transient contraction (Fig. 3). The pattern of contractions induced by Ang-III was similar to that observed with Ang-II. Median effective concentrations of Ang-I, II and III averaged 3.7, 0.8 and 2.5 × 10⁻⁷ M, respectively. Maximum tensions developed by 5 × 10⁻⁷ M Ang-I and III were always smaller than those by 10⁻⁷ M Ang-II. Equipotent doses of Ang-II to 5 × 10⁻⁷ M Ang-I and III were 2.9 and 9.1 × 10⁻⁸ M, respectively. The latency for inducing contractions in response to Ang-I, the time to peak tension and the half duration of contractions were significantly longer than those with Ang-II (Table 1). These parameters
obtained with Ang-II and III did not significantly differ, except that the half duration of contractions was shorter with Ang-III (Table 1).

Modification by blocking agents of the contractile response to angiotensins

Contractile responses of dog mesenteric arteries to Ang-I, II and III were suppressed
by treatment for 20 min with Sar² Ileu⁸ Ang-II (10⁻⁹ M) or Sar² Ala⁸ Ang-II (10⁻⁹ and 5 x 10⁻⁹ M) (Table 2). Percent inhibitions in the response to Ang-I, II and III were not significantly different (Table 2). The inhibitory effect of Sar² Ileu⁸ Ang-II was reversed only partially by repeated washing of preparations, but inhibition by Sar² Ala⁸ Ang-II was completely reversed. Contraction induced by 25 mM K⁺ was not influenced by 10⁻⁹ M Sar²

### Table 1. Contractile responses of dog mesenteric arteries to Ang-I, II and III

|                     | Ang-I             | Ang-II           | Ang-III          |
|---------------------|-------------------|------------------|------------------|
|                     | (5 x 10⁻⁷ M)      | (10⁻⁷ M)         | (5 x 10⁻⁷ M)     |
| Maximum tension (mg)*| 726 ± 70 (N=36)  | 874 ± 7.4 (N=36) | 955 ± 179 (N=6) |
|                     | (80 ± 2.5%a)      |                  | (90 ± 1.4%a)     |
| Latency for inducing contraction (sec) | 19.8 ± 1.9 (N=28) | 9.0 ± 1.0 (N=12) | 8.0 ± 1.0 (N=16) |
| Time to peak tension (sec) | 112 ± 9.8 (N=20) | 45.6 ± 1.9 (N=18) | 56.4 ± 3.6 (N=16) |
| Half duration of contraction (min) | 3.80 ± 0.33 (N=16) | 1.77 ± 0.20 (N=16) | 1.25 ± 0.07b (N=16) |

* N, number of preparations used. * Maximum tensions developed by Ang-I, II and III were compared in same preparations (Ang-I vs Ang-II, N=36; Ang-II vs Ang-III, N=6). a, Significantly different from the value with Ang-II, P<0.001. b, P<0.05.

### Table 2. Inhibition by angiotensin II antagonists of the response of mesenteric arteries to Ang-I, II and III

|                     | Ang-I             | Ang-II           | Ang-III          |
|---------------------|-------------------|------------------|------------------|
|                     | (5 x 10⁻⁷ M)      | (10⁻⁷ M)         | (5 x 10⁻⁷ M)     |
| Control             | 6 964 ± 207       | 8 754 ± 170      | 7 1040 ± 310     |
| Sar² Ileu⁸ Ang-II 10⁻⁹ M | 8 186 ± 97a (19.8 ± 6.1%a) | 8 180 ± 84b (19.7 ± 8.1%a) | 7 190 ± 109d (15.7 ± 9.7%a) |
| After wash          | 7 476 ± 185 (57.0 ± 5.4%a) | 8 444 ± 149 (52.9 ± 8.7%a) | 6 602 ± 256 (49.2 ± 7.0%a) |
| Control             | 6 1020 ± 234      | 8 906 ± 140      |                 |
| Sar² Ala⁸ Ang-II 10⁻⁹ M | 6 306 ± 78c (37.7 ± 13.8%a) | 8 242 ± 82b (28.4 ± 9.9%a) |                 |
| After wash          | 4 1030 ± 346 (98.7 ± 14.3%a) | 3 861 ± 210 (97.5 ± 19.3%a) |                 |
| Control             | 6 1064 ± 238      | 8 980 ± 138      |                 |
| Sar² Ala⁸ Ang-II 5 x 10⁻⁹ M | 6 102 ± 40a (12.7 ± 6.8%a) | 8 68 ± 38c (7.3 ± 4.7%a) |                 |
| After wash          | 4 1026 ± 520 (96.0 ± 23.0%a) | 7 844 ± 156 (87.1 ± 13.8%a) |                 |

N, number of preparations used. Figures in parentheses indicate relative values to controls. a, Significantly different from control, P<0.001. b, P<0.01. c, P<0.02. d, P<0.05.
Ileu^a Ang-II; mean values of the contraction before and after treatment with the antagonist were 836±241 mg and 846±232 mg (103±3.3% of control, N=4), respectively. The addition of the angiotensin antagonists in concentrations up to 10^{-6} M failed to produce contractions in isolated dog mesenteric arteries.

Contractions of mesenteric arterial strips induced by Ang-I, II and III were not significantly influenced by treatment with 10^{-6} M phentolamine, 10^{-6} M methysergide and 10^{-6} M diphenhydramine.

Treatment for 20 min with bradykinin-potentiator B (10^{-6} M) significantly attenuated

![Graph](image)

**Fig. 4.** Modification by bradykinin-potentiator B of the response of mesenteric arteries to Ang-I. Contractions induced by 5×10^{-7} M Ang-I in control media were taken as 100%; the mean absolute value was 1006±169 mg (N=13). a, Significantly different from control, P<0.001. b, P<0.01.

![Graph](image)

**Fig. 5.** Contractile responses of mesenteric arterial strips to Ang-I and II in the presence and absence of bradykinin-potentiator B. Two strips were obtained from the same dog. Arrows represent the addition of angiotensins.
the contractile effect of Ang-I (22.5±2.5% attenuation, N=13) and prolonged the half duration of contractions (Fig. 4). On the other hand, the response to Ang-II was not inhibited by the bradykinin-potentiator. Typical recordings of the responses to Ang-I and II in the presence and absence of the potentiator are demonstrated in Fig. 5.

**DISCUSSION**

In helically cut strips of dog mesenteric arteries, cross tachyphylaxis of the responses to Ang-I, II and III occurred and the responses were attenuated to a similar extent by Ang-II antagonists in concentrations in which the response to submaximum dose of K+ was unaffected. It may be concluded that the contractions induced by these angiotensins derive from activation of Ang-II receptors. Phentolamine, methysergide and diphenhydramine in concentrations sufficient to suppress the contractile response to the respective agonists failed to alter the effect of angiotensins in dog mesenteric arteries, as seen in isolated rabbit aortae (4) and rat portal veins (5), thus alpha-adrenergic, serotonergic and histaminergic H1 mechanisms are not involved in the genesis of angiotensin-induced contractions.

It has been demonstrated that Ang-II antagonists, Sar1 Ileu5 Ang-II and Sar1 Ala8 Ang-II, injected intravenously raise systemic blood pressure of dogs (6) and hypertensive patients (7) and reduce renal blood flow of rabbits (8) and dogs (9), probably by stimulation of Ang-II receptors. In the present study with isolated dog mesenteric arteries, however, the antagonists up to 10^{-6} M, 1,000 times higher than the dose sufficient to suppress the Ang-II action, failed to contract the arteries, as seen in isolated rat and rabbit aortae (8). Further, the pressor response of anesthetized dogs to Ang-II is suppressed by Ang-II antagonists in doses approx. the same as those of the agonists (10), whereas the response of isolated arterial strips to 10^{-7} M Ang-II was markedly attenuated by 10^{-8} M antagonists. We have no explanation as to why the agonistic action and the antagonistic potency of the antagonists in isolated and in situ preparations differ. Mimuran et al. (8) suggested that Sar1 Ala8 Ang-II failed to show an agonistic action in tissues which are relatively insensitive to Ang-II.

It has been demonstrated that Ang-III is less potent in causing vascular contractions than Ang-II but is more potent in stimulating aldosterone synthesis (3, 11). In the present study with dog mesenteric arterial strips, Ang-III was approx. 1/5 as potent as Ang-II, while in isolated rabbit thoracic aortae, the former peptide is approx. 1/60 as potent as the latter (12). Sar1 Ala8 Ang-II is as effective in blocking the pressor response of anesthetized rats to Ang-III as in inhibiting Ang-II (13). This was also the case in isolated dog mesenteric arteries.

The latency for inducing contractions and the time to peak tension developed by Ang-I were significantly longer than those with Ang-II and III. Further, the response to Ang-I was significantly attenuated by bradykinin-potentiator B, which inhibits a conversion of Ang-I to II (14, 15). These findings suggest that contractions induced by Ang-I result at least in part from a conversion to Ang-II in the arterial wall. A similar conclusion has been drawn by Aiken and Vane (2) who worked with arteries from dogs and rabbits. The presence
of the converting enzyme in rat aortae (16) and endothelial cells of pulmonary and umbilical vessels (15) has been demonstrated.

The concentration of Ang-II to give equal contractions induced by $5 \times 10^{-7}$ M Ang-I averaged $2.9 \times 10^{-8}$ M, the ratio of Ang-I to II is 17:1, which is approx. the same as that obtained with rabbit aortae (20:1) but is appreciably greater than with rabbit pulmonary arteries (4:1) (2). Bradykinin-potentiator B at $10^{-8}$ M attenuated the contraction induced by $5 \times 10^{-7}$ M Ang-I by 22.5%; it is estimated from the dose-response curve of Ang-II that in the presence of the converting enzyme inhibition, the equipotent dose of Ang-II to $5 \times 10^{-7}$ M Ang-I is $1.2 \times 10^{-8}$ M, the ratio being 42:1. Whether residual contractions in response to Ang-I after treatment with the inhibitor are due to the direct action of Ang-I on Ang-II receptors in arterial smooth muscles or to incomplete inhibition of the enzyme remains to be ascertained. Incubation for 15 to 20 min of plasma containing bradykinin-potentiator B in a concentration used in the present study ($10^{-6}$ M) inhibits the conversion of Ang-I to II almost completely (14, 15). If the latter is the case in isolated arterial smooth muscles and the diffusion rate and distribution for Ang-I and II are similar in such preparations, the bradykinin potentiator is estimated to inhibit the converting enzyme by 59%.

Acknowledgements: This study was supported in part by Scientific Research Fund (No. 248122) from the Ministry of Education, Science and Culture of Japan and also by the Takeda Medical Research Foundation.

REFERENCES
1) NG, K.K.F. AND VANE, J.R.: Conversion of angiotensin I to angiotensin II. Nature 216, 762–766 (1967)
2) AIKEN, J.W. AND VANE, J.R.: The renin-angiotensin system: inhibition of converting enzyme in isolated tissue. Nature 228, 30–34 (1970)
3) PEACH, M.W. AND ACKERLY, J.A.: Angiotensin antagonists and the adrenal cortex and medulla. Fedn Proc. 35, 2502–2507 (1976)
4) REGOLI, D., PAR, W.K. AND RIoux, F.: Pharmacology of angiotensin. Pharmacol. Rev. 26, 69–123 (1974)
5) BLAIR-WEST, J.R., McENZIE, J.S. AND MCKINLEY, M.J.: The actions of angiotensin II on the isolated portal vein of the rat. Europ. J. Pharmacol. 15, 221–250 (1971)
6) BUMPUS, F.M., SUN, S., SMEBY, R.R., SWEET, C., FERRARIO, C.M. AND KHOSLA, M.C.: Use of angiotensin II antagonists in experimental hypertension. Circulation Res. 32 and 33, Suppl. I, I-150-I-158 (1973)
7) CASE, D.B., WALLACE, J.M., KLEIN, H.J., SEALEY, J.E. AND LARAGH, J.H.: Usefulness and limitations of Saralasin, a partial competitive agonist of angiotensin II, for evaluating the renin and sodium factors in hypertensive patients. Am. J. Med. 60, 825–836 (1976)
8) MINURAN, A., HINRICHs, K.J. AND HOLLENBERG, N.K.: Characterization of smooth muscle receptors for angiotensin: studies with antagonist. Am. J. Physiol. 226, 185–190 (1974)
9) ABE, Y., KISHIMOTO, T. AND YAMAMOTO, K.: Effect of angiotensin II antagonist infusion on autoregulation of renal blood flow. Am. J. Physiol. 231, 1267–1271 (1976)
10) BRAVO, E.L., KHOSLA, M.C. AND BUMPUS, F.M.: Vascular and adrenocortical responses to a specific antagonist of angiotensin II. Am. J. Physiol. 228, 110–114 (1975)
11) CHU, A.T. AND PEACH, M.J.: Inhibition of induced aldosterone biosynthesis with a specific antagonist of angiotensin II. Proc. natn. Acad. Sci. U.S.A. 71, 341–344 (1974)
12) MOORE, A.F., HALL, M.M. AND KHAIRALLAH, P.A.: A comparison of the effects of angiotensin II and heptapeptide on smooth muscle (vascular and uterine). Europ. J. Pharmacol. 39, 101–107 (1976)
13) SPIELMAN, W.S., DAVIS, J.O. AND FREEMAN, R.H.: Des-Asp-l-angiotensin II: possible role in mediating the renin-angiotensin response in the rat. Proc. Soc. exp. Biol. Med. 151, 177–182 (1976)

14) OGISHARA, T., YAMAMOTO, T., DOI, K., KUMAHARA, Y., KIMURA, T. AND SAKAKIBARA, S.: In vitro study on the effects of bradykinin potentiating factor and angiotensin II analogue on degradation and conversion of angiotensin I and II in plasma. Clin. Chim. Acta 52, 287–292 (1974)

15) UEDA, E., KOKUBU, T., AKUTSU, H. AND YAMAMURA, Y.: Inhibition of angiotensin I converting enzyme and kininase in rabbit plasma by bradykinin potentiating peptide B (Pyr-Gly-Leu-Pro-Arg-Pro-Lys-Ile-Pro-Pro). Experientia 27, 1020–1021 (1971)

16) CUSHMAN, D.W. AND CHEUNG, H.S.: Concentrations of angiotensin converting enzyme in tissues of the rat. Biochim. Biophy. Acta 250, 261–265 (1971)

17) ERDÖS, E.G.: Conversion of angiotensin I to angiotensin II. Am. J. Med. 60, 749–759 (1976)