Pharmacological Activity of Angiotensin-II Modified by Tyrosine Sulfation

Masaki HAGIWARA, Eiko OHUCHI, Kazuya HONGO, Miyuki OKI, Koichi WADA, Tadanori MORIKAWA and Kyoichi KOBASHI

Fuji Chemical Industries, Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan
1 Department of Biochemistry, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

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Abstract—An angiotensin-II analogue with a sulfated tyrosine residue was prepared by arylsulfotransferase treatment of synthetic human angiotensin-II. Its biological activities were studied in isolated smooth muscles, and its effect on blood pressure was determined. The sulfated angiotensin-II (All-S) was about 15–30 fold less potent than angiotensin-II (All) for ileum contraction and gallbladder contraction. The hypertensive potency of All-S was about 30-fold less than that of All.

Angiotensin-II is an endogenous vasoactive octapeptide that is involved in the regulation of blood pressure and acts on extrascular smooth muscles such as guinea pig ileum and rat uterus in vitro. Many synthetic analogues of angiotensin have been prepared and tested to clarify the relationship between its chemical structure and biological activities (1). However, the influence of tyrosine sulfation in angiotensin on its activities is yet unknown. Marked alteration of biological activity by sulfation of a tyrosine residue has been reported for CCK and hirudin. CCK requires tyrosine sulfation for its action (2–5), and tyrosine sulfation of synthetic hirudin peptide increases its anticoagulant activity (6).

Arylsulfotransferase (AST) obtained from Eubacterium A-44, which is a predominant bacterium in human intestine, has been reported to catalyze the transfer of a sulfate group from phenolic esters to other phenolic compounds with strict specificity (7-9). It has been found that the tyrosine of some peptides, such as enkephalin, LH-RH, vasopressin, angiotensin and proctolin, were specifically sulfated by AST with high yields (9).

In order to determine how tyrosine sulfation of angiotensin-II affects its biological activities, the fourth residue, tyrosine, of human angiotensin-II (All) was sulfated by AST, and several biological activities of the sulfated angiotensin-II (All-S) were examined in vitro and in vivo in comparison with All.

All was purchased from the Peptide Institute, Inc. (Osaka, Japan). All was enzymatically sulfated by AST according to the methods described by Kobashi et al. (9). The purity and structure were ascertained by TLC and amino acid analysis. The purity of All-S was more than 99%. All and All-S were dissolved in saline solution.

Male Hartley guinea pigs, weighing 300–400 g, were killed by a blow on the head after 24 hr fasting. The ileum and the gallbladder were removed and placed in Tyrode’s solution containing: NaCl, 8.0; CaCl2, 0.2; KCl, 0.2; MgCl2, 0.071; NaH2PO4, 0.05; glucose, 1.0; and NaHCO3, 1.0 (g/l), or in modified Locke-Ringer solution (low calcium) containing: NaCl, 8.8; CaCl2, 0.04; KCl, 0.4; MgCl2, 0.018; Na2HPO4, 0.08; KH2PO4, 0.08; glucose, 0.5; and NaHCO3, 0.4 (g/l).

The terminal portion of 20 mm was dissected from the ileum and was mounted in a 15-ml organ bath, containing Tyrode’s solution bubbled with air and maintained at 32±0.5°C. The tissue was placed initially under a
resting tension of 1 g and allowed to equilibrate for 30 min. The contraction of the ileal preparations was measured using a KN-259 isotonic transducer (Matume, Tokyo).

The gallbladder was cut into a 15–20-mm long, 2–3-mm wide strip. The preparation was mounted in a 15-ml organ bath containing low calcium Locke-Ringer solution bubbled with air and maintained at 32±0.5°C. A resting tension of 0.6–0.7 g was loaded to the preparations. The contraction was measured using a Model IM-300 isometric transducer (Fujiotec, Tokyo).

After confirmation of stable contractile responses of these preparations to repeated stimulation with acetylcholine (1.65×10⁻⁶ M) to the ileum, or CCK-8 (10⁻⁸ M) to the gallbladder, the contractile response to All or All-S was estimated. The maximal contraction induced by 1.65×10⁻⁶ M acetylcholine and 10⁻⁸ M CCK-8S was regarded as a reference response (100%) in the ileum and the gallbladder, respectively.

In order to assess the effect of All-S on blood pressure, male Wistar rats, weighing 300–400 g, were anesthetized with urethane (1.25 g/kg, i.p.). Blood pressure was measured with a pressure transducer through a polyethylene cannula inserted into the carotid artery and recorded on a polygraph (Nihon Kohden, Tokyo). The peptides were injected into the femoral vein.

Results are expressed as means±S.E. The significance of results was evaluated using Student’s unpaired t-test. The relative potency was computed using a parallel line assay (10).

The contractile effects of All-S and All on isolated rat ileum preparations are shown in Table 1. These peptides produced contractions of the preparation in a concentration-dependent manner. The activity of All was higher than that of All-S, and the potency ratio was 15 (7–31, 95% conf. limits).

Gallbladder strips were also contracted by All-S (10⁻⁷ to 10⁻⁶ M) in a concentration-dependent manner (Table 1). However, The All-S was less potent than All. At the concentration (10⁻⁷ M) at which All showed the maximal response, All-S produced less than 10% contraction. The potency ratio of All was 37 (18–74, 95% conf. limits).

All-S or All, when administered intravenously, produced immediately a short lasting (3–5 min), but significant increase in blood pressure in a dose-dependent manner. No difference in duration and time to peak (0.5–1 min) was observed between the hypertensive responses to All-S and All. Figure 1 shows dose-response curves of All and All-S for their hypertensive effects. All-S appeared to be about 30 times less potent in the hypertensive response than All. Heart rate was decreased by injection of All or All-S. Mean decreased heart rate by All and All-S were 40 and 15 beats/min, at the dose of 10⁻⁸ mol/kg, respectively.

Table 1. Relationships between concentration and response for contraction in the ileum and the gallbladder of guinea pigs

| Compounds | Concentration M | ileum% | N | gallbladder% | N |
|-----------|----------------|--------|---|-------------|---|
| All       | 10⁻⁹           | 21.7±6.1 | 3 | 3.2±2.3     | 3 |
|           | 3×10⁻⁹         | 39.9±3.9 | 3 | 22.8±2.6    | 3 |
|           | 10⁻⁸           | 73.7±5.5 | 3 | 55.2±5.3    | 3 |
|           | 3×10⁻⁸         | 85.0±5.1 | 3 | 90.3±3.7    | 3 |
|           | 10⁻⁷           | 24.7±4.1 | 3 | 9.2±5.0     | 3 |
| All-S     | 10⁻⁹           | 36.3±4.4 | 3 |             |   |
|           | 3×10⁻⁹         | 53.8±5.1 | 3 |             |   |
|           | 10⁻⁷           | 19.5±3.0 | 3 |
|           | 3×10⁻⁷         | 50.5±4.4 | 3 |

Maximum contraction by 1.65×10⁻⁶ M Ach (a) and 10⁻⁸ M CCK-8 (b) was regarded as a reference response (100%) in the ileum and the gallbladder, respectively.
Fig. 1. Pressure response to sulfated (All-S) and non-sulfated (All) angiotensin-II in anesthetized rats. Data indicate an increased pressure response (mmHg) after the injection. N=3, *P<0.01 vs. All.

The present study clearly showed that sulfation of the fourth amino acid residue of All tyrosine, decreased the biological action of All. Such a decrease in biological action by tyrosine sulfation was reported to occur in leu-enkephalin (11). In contrast with these peptides, CCK requires tyrosine sulfation for its full biological activity, and the anticoagulant activity of hirudin was potentiated by sulfation of its tyrosine (2-6). This study provides evidence that the fourth amino acid of All tyrosine could influence the active site of All, possibly by affecting its binding to the receptors. It has been reported that replacement of tyrosine with alanine reduced the affinity of All to the rat stomach strips (1).

Sulfation of tyrosine might influence the cleavage of All in the body. However, reduction of the activity by sulfation of All was observed in vivo blood pressure response, and no difference in duration and peak time of pressure response between All and All-S were demonstrated in this study. These suggest that the weaker potency of All-S compared with All is not attributable to the metabolic rate of the peptide.

Further studies are required to clarify the biological significance of the sulfation of All.

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