EFFECT OF [Asu\textsuperscript{1-7}]-EEL CALCITONIN AND ITS FRAGMENTS ON GASTRIC ACID SECRETION IN RATS

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Accepted March 6, 1981

Abstract—Effect of [Asu\textsuperscript{1-7}]-eel calcitonin ([Asu\textsuperscript{1-7}]-E-CT), an analogue of natural eel calcitonin, and its fragments on gastric acid secretion was studied in pylorus-ligated rats. [Asu\textsuperscript{1-7}]-E-CT markedly reduced the acid secretion with intravenous and intramuscular doses of 3 Medical Research Council units (MRC U)/kg or 0.6 \( \mu \text{g/kg} \). This activity was comparable to that of salmon calcitonin and porcine calcitonin compared with equivalent hypocalcemic doses and was consistently evident during daily administrations for 10 days. Assay of six fragments of [Asu\textsuperscript{1-7}]-E-CT showed that the C-terminal peptide consisting of 22 amino acids diminished the acid secretion but shorter peptides of the C-terminal fragment or the N-terminal fragments did not. These results suggest that the C-terminal peptide may play an important role in this action of [Asu\textsuperscript{1-7}]-E-CT, and a peptide chain of over 18 amino acids is required.

Calcitonin is considered to be a hypocalcemic hormone existing in the ultimobranchial body or thyroid of eel, salmon, porcine, human and other species. However, the physiological role is probably not merely hypocalcemic. Many investigators reported that exogenous (1-7) and endogenous (8) calcitonin reduced gastric acid secretion in experimental animals.

[Asu\textsuperscript{1-7}]-eel calcitonin ([Asu\textsuperscript{1-7}]-E-CT) is a synthetic polypeptide possessing hypocalcemic activity comparable to that of natural eel calcitonin and higher stability than the natural product (9-11). It was synthesized by replacing the N-terminal amino acid and the s-s bond of the natural eel calcitonin with a hydrogen atom and an ethylene linkage of L-amino suberic acid, respectively (9).

This paper describes the effects of [Asu\textsuperscript{1-7}]-E-CT and its fragments on gastric acid secretion in rats.

MATERIALS AND METHODS

The pylorus ligation was performed following the procedure of Shay et al. (12) under light anesthesia with ether. Male Wistar rats fasted for 24 hours were used and test compounds were administered immediately after ligation. After 4 hours, the rats were again anesthetised, the gastric contents collected in test tubes and the volume of supernatant quantified after centrifugation at 1500 r.p.m. for 5 minutes. The acid concentration (mEq/l) was measured by titration with 0.2 N NaOH to pH 7.4 using a titrator (Radiometer, TTT 1c) and a syringe burette (Radiometer, SBU 1a). The acid output (\( \mu \text{Eq/hr} \)) was calculated from the volume and the acid concentration.
Levels of statistical significance for group differences were determined using Student's t-test. P values of 0.05 were considered significant.

The drugs used were: [Asu₁⁻⁷]-E-CT (Toyo Jozo, 6000 MRC U/mg*), fragment [Asu₁⁻⁷] 1-11, [Asu₁⁻⁷] 1-18, 11-32, 15-32, 20-32 and 26-32 (Toyo Jozo), salmon calcitonin (Sandoz, 4000 MRC U/mg*) and porcine calcitonin (UCLAF, 0.22 MRC U/mg*). The chemical structure of [Asu₁⁻⁷]-E-CT and its fragments is shown in Fig. 1. The drugs were dissolved in 0.5% citrate buffer, pH 6.0, containing 0.2% gelatine.

**RESULTS**

1. Effect of [Asu₁⁻⁷]-E-CT administered intramuscularly: Effect of [Asu₁⁻⁷]-E-CT i.m. on gastric acid secretion is shown in

* Unitage is based on the 1-hr hypocalcemic activity in the rats of the test preparation administered intravenously, compared to the Medical Research Council (MRC) salmon calcitonin.

Table 1. [Asu₁⁻⁷]-E-CT in a dose of 3 MRC U/kg decreased significantly the volume of gastric juice and the gastric acid output and lowered the acid concentration in a dose of 10 MRC U/kg. This inhibitory activity was dose dependent.

[Asu₁⁻⁷]-E-CT was given i.m. daily for 3 and 10 consecutive days and the effect on the acid secretion was examined on the third and tenth days. [Asu₁⁻⁷]-E-CT diminished the volume and the titratable acidity of the gastric juice, dose dependently. This activity was similar to that observed in the single injection experiment.

2. Comparison of effect of intravenously administered [Asu₁⁻⁷]-E-CT, salmon calcitonin and porcine calcitonin: The inhibitory activity of [Asu₁⁻⁷]-E-CT on the acid secretion was compared with that of salmon calcitonin and porcine calcitonin (Table 2). [Asu₁⁻⁷]-E-CT reduced the acid secretion dose dependently and the minimum effective dose was 3 MRC U/kg. A similar dose response
Table 1. Effect of [Asu^1-7]-eel calcitonin on the gastric acid secretion in pylorus ligated rats.

| Treatment (MRC U/kg) i.m. | No. | Body weight (g) | Gastric secretion |
|---------------------------|-----|-----------------|-------------------|
|                           |     |                 | Volume (ml)       | Acid concentration (mEq/l) | Acid output (μEq/hr) |
| Single administration     |     |                 |                   |                            |                     |
| 0                         | 7   | 144.6± 5.3      | 3.6±1.1           | 110.0± 8.1                 | 99.1±30.4           |
| 1                         | 7   | 145.1± 3.4      | 3.2±1.0           | 102.5±11.9                | 83.7±34.7           |
| 3                         | 7   | 146.3± 5.1      | 1.6±0.7*          | 99.3±12.0                 | 39.5±19.5*          |
| 10                        | 7   | 143.7± 4.2      | 0.8±0.3*          | 65.2± 9.8                 | 13.3± 5.7*          |
| Daily administrations for 3 days |    |                 |                   |                            |                     |
| 0                         | 7   | 162.0±12.9      | 4.9±1.3           | 112.3±11.7                | 138.1±44.1          |
| 3                         | 7   | 172.9± 9.9      | 1.9±0.4*          | 92.1±12.6*                | 44.1±13.5*          |
| 10                        | 7   | 176.3±23.9      | 0.6±0.4*          | 71.3±15.6*                | 10.6± 8.1*          |
| Daily administrations for 10 days |   |                 |                   |                            |                     |
| 0                         | 6   | 188.6±17.3      | 6.0±1.3           | 104.6±10.5                | 157.0±37.0          |
| 3                         | 7   | 186.4±11.2      | 2.1±1.2*          | 91.3±11.1*                | 49.9±31.7*          |
| 10                        | 7   | 173.0±20.3      | 0.8±0.5*          | 60.9± 9.4*                | 12.4± 8.5*          |

Each value shows mean±S.D.
*: Significant difference from control (p=0.05)

Table 2. Comparison of effect of [Asu^1-7]-eel calcitonin, salmon calcitonin and porcine calcitonin on the gastric acid secretion in pylorus ligated rats.

| Treatment (MRC U/kg) i.v. | No. | Body weight (g) | Gastric secretion |
|---------------------------|-----|-----------------|-------------------|
|                           |     |                 | Volume (ml)       | Acid concentration (mEq/l) | Acid output (μEq/hr) |
| [Asu^1-7]-eel calcitonin  |     |                 |                   |                            |                     |
| 0                         | 6   | 145.3± 5.8      | 4.4±0.7           | 93.7±10.7                 | 103.2±25.0          |
| 1                         | 6   | 146.0±7.8       | 3.5±0.8           | 101.9±11.3                | 89.5±25.8           |
| 3                         | 6   | 144.8±6.6       | 1.3±0.6*          | 71.7±17.0*                | 25.5±16.9*          |
| 10                        | 6   | 149.3±5.7       | 0.7±0.1*          | 32.4±13.7*                | 5.7± 3.1*           |
| 30                        | 6   | 143.3±5.2       | 0.3±0.1*          | 30.7±25.5*                | 2.1± 1.8*           |
| Salmon calcitonin         |     |                 |                   |                            |                     |
| 0                         | 6   | 121.0±5.4       | 3.6±0.8           | 97.2± 4.3                 | 86.5±18.3           |
| 1                         | 6   | 124.0±5.1       | 3.3±0.7           | 102.3± 4.1                | 84.9±19.5           |
| 3                         | 6   | 122.0±2.1       | 1.1±0.3*          | 40.4±26.2*                | 11.3± 8.9*          |
| 10                        | 6   | 119.5±6.1       | 0.4±0.2*          | 61.2±10.2*                | 5.0± 2.0*           |
| 30                        | 6   | 122.7±5.4       | 0.3±0.1*          | 54.3±16.7*                | 3.7± 1.8*           |
| Porcine calcitonin        |     |                 |                   |                            |                     |
| 0                         | 6   | 128.7±5.5       | 3.4±0.6           | 99.9± 2.1                 | 84.4±15.8           |
| 1                         | 6   | 128.3±8.4       | 2.0±0.8*          | 92.6±14.3                 | 47.8±22.3*          |
| 3                         | 6   | 130.0±8.7       | 1.3±0.7*          | 64.7±24.4*                | 19.1±13.9*          |
| 10                        | 6   | 126.0±5.2       | 0.8±0.2*          | 54.8±28.3*                | 9.6± 5.1*           |
| 30                        | 6   | 125.7±5.6       | 0.4±0.1*          | 60.2± 6.8*                | 5.7± 1.7*           |

Each value shows mean±S.D.
*: Significant difference from control (p=0.05)
A curve was obtained with salmon calcitonin but porcine calcitonin at equivalent hypocalcemic dosages (Fig. 2). Porcine calcitonin diminished the volume and the acid output even at a dose of 1 MRC U/kg, though the activity at doses of 3 to 30 MRC U/kg was similar to that of [Asu₁⁻⁷]-E-CT and that of salmon calcitonin.

3. Effect of fragments of [Asu₁⁻⁷]-E-CT: Effect of subcutaneous administration of [Asu₁⁻⁷]-E-CT and its fragments on the gastric acid secretion are shown in Table 3. The fragment 11-32 significantly reduced the volume and the acid output at a dose of $5.95 \times 10^{-9}$ mol/kg and lowered the acid concentration at a dose of $5.95 \times 10^{-8}$ mol/kg. However, other fragments, [Asu₁⁻⁷]-1-11, [Asu₁⁻⁷]-1-18, 15-32, 20-32 and 26-32, failed to affect the acid secretion at doses as large as $5.95 \times 10^{-8}$ mol/kg. [Asu₁⁻⁷]-E-CT markedly decreased the acid secretion even at a dose of $5.95 \times 10^{-10}$ mol/kg, such being fairly equivalent to the dosage of 10 MRC U/kg.

DISCUSSION

[Asu₁⁻⁷]-E-CT diminished the volume and the titratable acidity of the gastric juice when given intramuscularly, intravenously and subcutaneously. There was little difference among the activities on the acid output with any of these routes. The minimum effective dose was 3 MRC U/kg which corresponded to 0.6 μg/kg. This activity was consistently observed during daily administrations of [Asu₁⁻⁷]-E-CT for 3 and 10 days. This suggests that neither tolerance nor sensitization to the gastric acid secretion may occur during administrations of [Asu₁⁻⁷]-E-CT for a long period of time.

The inhibitory activity of [Asu₁⁻⁷]-E-CT was similar to that of salmon calcitonin at doses of 1 to 30 MRC U/kg, but somewhat less potent than that of porcine calcitonin at a dose of 1 MRC U/kg. Bobalik et al. (4) stated that porcine and salmon calcitonin had equipotent activity in the inhibition of gastric acid secretion in equivalent hypocalcemic dosages. The discrepancy with our findings may be related to the purity of porcine calcitonin; the purity of the compound they used was 62 MRC U/mg while that of our product was 0.22 MRC U/mg.

Sieber et al. (13) reported that fragments of porcine calcitonin did not have hypocalcemic activity and that the C-terminal amino group is necessary for the biological activity. Rittel et al. (14) further showed that opening of the disulfide bond in 1,7 amino acids and deletion of the C-terminal
Table 3. Effect of [Asu1-7]-eel calcitonin ([Asu1-7]-E-CT) and its fragments on the gastric acid secretion in pylorus ligated rats.

| Treatment (mol/kg, s.c.) | No. | Body weight (g) | Volume (ml) | Acid concentration (mEq/l) | Acid output (uEq/hr) |
|--------------------------|-----|-----------------|-------------|----------------------------|---------------------|
| Control                  | 5   | 144.7±9.1       | 5.1±1.3     | 108.2±5.0                  | 138.1±38.0          |
| 11–32 5.95×10^{-10}      | 6   | 142.8±5.7       | 4.8±1.1     | 106.9±15.1                 | 131.3±43.5          |
| 11–32 5.95×10^{-9}       | 6   | 146.8±9.3       | 2.1±0.5*    | 91.5±17.2                  | 49.0±19.1*          |
| 11–32 5.95×10^{-8}       | 5   | 148.0±8.9       | 0.7±0.5*    | 56.9±2.8*                  | 10.3±7.0*           |
| [Asu1-7]-E-CT 5.95×10^{-10}** | 6   | 144.0±7.2       | 1.1±0.2*    | 56.8±11.7*                 | 15.6±5.8*           |
| Control                  | 6   | 158.8±2.4       | 4.7±0.9     | 98.2±6.6                   | 116.7±27.9          |
| [Asu1-7]-1–11 5.95×10^{-10} | 6   | 157.0±9.7       | 4.9±1.0     | 100.4±4.0                  | 122.6±29.3          |
| [Asu1-7]-1–11 5.95×10^{-9} | 6   | 155.0±5.2       | 5.1±0.8     | 102.1±6.1                  | 130.2±25.1          |
| [Asu1-7]-1–11 5.95×10^{-8} | 6   | 159.7±6.7       | 4.8±0.3     | 93.0±7.2                   | 110.6±11.8          |
| [Asu1-7]-E-CT 5.95×10^{-10}** | 6   | 161.8±5.1       | 0.8±0.1*    | 46.9±21.1*                 | 8.9±4.3*            |
| Control                  | 6   | 137.8±5.0       | 4.2±0.6     | 94.3±13.1                  | 99.6±29.1           |
| [Asu1-7]-1–18 5.95×10^{-10} | 6   | 140.2±7.2       | 4.4±0.8     | 104.9±5.4                  | 114.7±26.2          |
| [Asu1-7]-1–18 5.95×10^{-9} | 5   | 142.0±9.8       | 5.3±0.6     | 102.4±7.2                  | 135.2±22.0          |
| [Asu1-7]-1–18 5.95×10^{-8} | 5   | 136.2±5.6       | 4.0±1.2     | 102.7±2.8                  | 103.8±33.7          |
| [Asu1-7]-E-CT 5.95×10^{-10}** | 5   | 137.2±5.7       | 0.8±0.4*    | 69.7±6.1*                  | 14.6±6.2*           |
| Control                  | 6   | 166.0±3.6       | 4.8±1.1     | 114.3±6.4                  | 137.1±33.5          |
| 15–32 5.95×10^{-10}      | 6   | 163.8±5.1       | 4.7±1.0     | 117.7±9.4                  | 132.0±33.6          |
| 15–32 5.95×10^{-9}       | 6   | 164.5±8.7       | 5.1±0.7     | 104.1±6.9                  | 131.0±18.0          |
| 15–32 5.95×10^{-8}       | 5   | 160.2±5.1       | 5.2±0.4     | 109.5±6.3                  | 142.9±12.8          |
| [Asu1-7]-E-CT 5.95×10^{-10}** | 5   | 163.0±7.0       | 0.8±0.2*    | 41.0±11.4*                 | 7.8±3.0*            |
| Control                  | 6   | 146.8±7.1       | 4.2±1.2     | 80.8±4.3                   | 85.3±26.3           |
| 20–32 5.95×10^{-10}      | 6   | 153.7±9.3       | 4.3±0.8     | 84.8±4.3                   | 91.1±17.0           |
| 20–32 5.95×10^{-9}       | 6   | 153.0±5.9       | 5.8±1.2     | 90.3±9.0                   | 131.8±34.6          |
| 20–32 5.95×10^{-8}       | 6   | 147.7±6.1       | 4.9±1.6     | 92.2±10.2                  | 114.3±41.7          |
| [Asu1-7]-E-CT 5.95×10^{-10}** | 6   | 150.2±8.4       | 0.9±0.3*    | 15.4±13.2*                 | 3.5±3.5*            |
| Control                  | 6   | 143.8±4.7       | 4.7±0.8     | 94.6±7.7                   | 110.9±23.0          |
| 26–32 5.95×10^{-10}      | 6   | 146.5±7.7       | 5.0±1.3     | 94.0±7.1                   | 119.1±33.0          |
| 26–32 5.95×10^{-9}       | 6   | 141.0±3.3       | 5.0±1.4     | 96.7±8.5                   | 119.5±32.0          |
| 26–32 5.95×10^{-8}       | 6   | 140.7±8.1       | 4.9±1.0     | 98.8±7.0                   | 121.8±26.2          |
| [Asu1-7]-E-CT 5.95×10^{-10}** | 6   | 144.2±4.0       | 0.5±0.2*    | 36.7±21.5*                 | 4.7±2.9*            |

Each value shows mean±S.D.

*: Significant difference from control (p<0.05)  **: [Asu1-7]-E-CT 5.95×10^{-10} mol/kg=10 MRCa/kg

amino group of human calcitonin resulted in loss of the hypocalcemic activity. In our present study, six fragments of [Asu1-7]-E-CT were examined as to their effects on gastric acid secretion. The fragment 11–32, C-terminal peptide of [Asu1-7]-E-CT, diminished the acid secretion, though the activity was ten to hundred fold less potent than that of [Asu1-7]-E-CT, at a equivalent molar dose (Table 3). The fragments 15–32, 20–32 and 26–32, which are shorter C-terminal fragments than the fragment 11–32 (Fig. 1) did not affect the acid secretion, even at high doses. The fragment [Asu1-7]
1-11 and [Asu¹,7⁻¹] 1-18, N-terminal peptide, did not have the activity. The fragment [Asu¹,7⁻¹] 1-18 has the same amino acid sequence, Lys¹⁻Leu-Ser-Gln-Glu-Leu-His-Lys¹⁸, as the fragment 11-32 in the molecule (Fig. 1), but nevertheless clear-cut activity. These results suggest that the C-terminal peptide might play an important role in the inhibitory effect on the acid secretion, and that a peptide chain of more than 22 amino acids close to C-terminal is required for the high potency. Sieber et al. (13) and Rittel et al. (14) reported that the entire structure of calcitonin was indispensable for the production of the biological activities. Our presently reported results showed that the C-terminal fragment of [Asu¹,7⁻¹]-E-CT (fragment 11-32) had an inhibitory activity on the acid secretion.

Acknowledgement: We thank Prof. K. Watanabe (Toyama Medical and Pharmaceutical University) for reviewing the manuscript and for pertinent advice.

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