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# EVIDENCE FOR THE PRESENCE OF DIPEPTIDYL CARBOXYPEPTIDASE AND ITS INHIBITORS IN INFLAMMATORY SYNOVIAL FLUIDS

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## Summary

Evidence is reported for the presence of dipeptidyl carboxypeptidase (peptidyldipeptide hydrolase, EC 3.4.15.1) and of inhibitor(s) of this enzyme in synovial fluids from patients with rheumatoid arthritis and gout.

Dipeptidyl carboxypeptidase (peptidyldipeptide hydrolase, EC 3.4.15.1) is a well known enzyme which converts angiotensin I to angiotensin II and inactivates bradykinin [1,2].

The enzyme has been found bound to the luminal surface of endothelial cells as well as in the plasma [3]. Alterations of enzyme activity in blood have been found in some diseases [4—6].

High kinin levels have been observed in synovial fluids from patients with various forms of arthritis and it has been suggested that these polypeptides may contribute to the inflammatory synovial reaction [7—9]. In particular a bradykinin concentration higher than $1 \cdot 10^{-9}$ M has been found in 40% of the synovial fluids from patients with rheumatoid arthritis [9] and from some patients with gout [7,8].

This paper reports evidence for the presence of dipeptidyl carboxypeptidase and its inhibitor(s) in synovial fluids from patients with inflammatory joint diseases.

SQ 20881, a high specific nonapeptide inhibitor of dipeptidyl carboxypeptidase [10,11], and Z-Phe-His-Leu substrate for the enzyme were obtained

from Serva A.G. Dipeptidyl carboxypeptidase was purified from bovine kidney cortex [12]. All other reagents were high purity commercial samples from Merck A.G.

Enzyme activity was measured by a fluorimetric method according to published procedures [13]. The reaction mixture contained 0.05 M Tris-HCl, pH 7.4/0.2 M NaCl/5 · $10^{-5}$ M Z-Phe-His-Leu/0.01—0.1 ml synovial fluid in a final volume of 2 ml. All incubations were conducted at 37°C and the substrate (1 mg/ml Z-Phe-His-Leu in methanol) was added last to initiate the reaction. After 1 h (data reported in Fig. 1) or 2 h incubation (data reported in Table I), the reaction was terminated by addition of 0.4 ml 2 M NaOH. 1 ml water, 0.1 ml 1% *o*-phtalaldehyde and, after 6 min, 0.2 ml 6 N HCl were added to the mixture. The precipitated protein was removed by centrifugation and the supernatant was used for fluorescence measurements.

The blanks, containing 0.05 M Tris-HCl, pH 7.4/0.2 M NaCl/the required amounts of synovial fluid, were incubated simultaneously with the samples at 37°C. The substrate was added in the blank solution after inactivation of the enzyme by 2 M NaOH.

Fluorescence was measured at an excitation wavelength of 370 nm and an emission wavelength of 500 nm in an Aminco-Bowman spectrophotofluorimeter with 10 × 10 mm cell and right-angle optical geometry. Fluorescence of a standard solution of His-Leu (10 nmol) was measured in duplicate, simultaneously with that of samples and blanks.

Synovial fluid was obtained from two patients with rheumatoid arthritis (patient A and B) and from one patient with gout (patient C).

Rheumatoid arthritis was diagnosed according to the criteria of the American Rheumatism Association [14]. The diagnosis of gout was based on the finding of urate crystals in the synovial fluid [15].

Two specimens of synovial fluid were obtained from patient A: the first one, aspirated from the right knee, contained 11 000 white cells/$mm^3$ (74% neutrophils). The second one, aspirated from the left wrist, contained 9800 white cells/$mm^3$ (60% neutrophils). Synovial fluids from patient B and C were obtained from the knee joint and contained 11 200 white cells/$mm^3$ (66%

TABLE I

PEPTIDYL DIPEPTIDASE ACTIVITY IN SYNOVIAL FLUIDS

Synovial fluid activity was measured using 20 μl rheumatoid fluids or 50 μl gouty synovial fluid in a reaction mixture containing: 0.05 M Tris-HCl, pH 7.4/0.2 M NaCl/50 μM Z-Phe-His-Leu in a final volume of 2 ml. Incubation temperature was 37°C.

| Patient | Diagnosis | Joint | nmol His-Leu formed/h per mol synovial fluid | |
|---|---|---|---|---|
| | | | in absence of SQ20881 | in presence $1 \cdot 10^{-5}$ M SQ20881 |
| A | Rheumatoid arthritis | wrist | 165 | n.d. |
| A | Rheumatoid arthritis | knee | 380 | 0 |
| B | Rheumatoid arthritis | knee | 230 | 0 |
| C | Gout | knee | 316 | 0 |

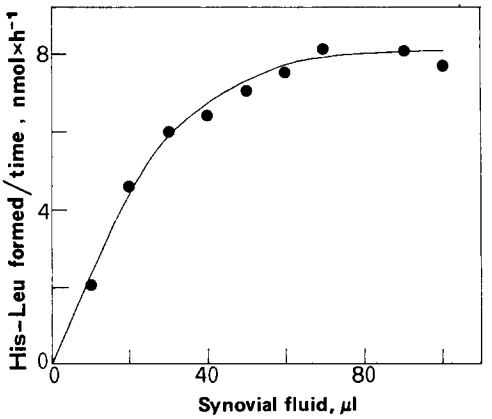


Fig. 1. Plot of the initial velocity vs. amount of synovial fluid (from the knee of patient B) added to the reaction mixture. The experimental conditions were as described in Table I.

neutrophils) and 12 000 white cells/mm$^3$ (88% neutrophils), respectively. No red cells were present in the fluids.

Heparinized synovial fluids were centrifuged to remove cells and stored either at 4°C, if assayed in the same day, or at —20°C if used in the following days. Synovial fluids were frozen only once and thawed immediately before use since a variation of dipeptidyl carboxypeptidase activity was observed after repeated freeze-thawing. All assays were performed within a week from arthrocentesis.

Table I shows the dipeptidyl carboxypeptidase activity of synovial fluids in the absence and in the presence of SQ 20881. The absence of enzyme activity in the presence of SQ 20881, which is a highly specific inhibitor of dipeptidyl carboxypeptidase [10,11], suggests that the hydrolysis of Z-Phe-His-Leu is carried out by this enzyme only.

As shown in Fig. 1, the plot of initial velocity vs. amount of synovial fluid (from the knee joint of patient B) added to the reaction mixture was not linear; this suggests that inhibitor(s) is (are) present in the synovial fluid [16]. The same result was obtained using the synovial fluids aspirated from the knee joint of the two other patients. The presence of inhibitor(s) in synovial fluids was confirmed by the following observation. In the presence of synovial fluid the activity of purified dipeptidyl carboxypeptidase was lower than the sum of the activities of the synovial fluid and the purified enzyme preparation measured under the same experimental conditions. Using 0.025 ml synovial fluids aspirated from the knee of patients A and B, and 0.19 mU purified dipeptidyl carboxypeptidase in a final volume of 1 ml, a 28 and 40% inhibition was observed, respectively.

Taking into account that bradykinin concentration in inflammatory synovial fluids is lower than $5 \cdot 10^{-8}$ M [8,9] and that, in the dipeptidyl carboxypeptidase assays, fluids were diluted at least 20-fold, the presence of bradykinin cannot fully explain the observed inhibition [17]. Therefore, other inhibitors must be present in synovial fluids.

The data obtained lead to the conclusion that dipeptidyl carboxypeptidase and inhibitor(s) of this enzyme are very probably present in inflammatory synovial fluids. The possibility that a modulation of the enzyme activity might have a role in the degree of joint inflammation must be considered.

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