AN UNCOMMON INOTROPICALLY ACTIVE PEPTIDE FROM DOG BLOOD PLASMA

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Summary

An octapeptide possessing inotropic activity has been isolated from heparinised dog blood plasma. It was found to be composed of seven isoleucine and one glycine residues and an as yet unidentified compound (X). The aminoacid sequence of its peptide chain was elucidated as iLeu. iLeu. iLeu, iLeu. Gly. iLeu. iLeu. The effect of this peculiar peptide compound on the dog heart papillary muscle was found to parallel that of adrenaline calculated on a Mole/Mole basis. The activity of the peptide compound was destroyed by mineral acid hydrolysis.

The presence in the blood plasma of pharmacologically active compounds of peptide nature has been known for quite a long time. Some of them 'kinins' have been isolated, structurally characterised and their pharmacodynamic action carefully studied (for review see Pierce (1) and Hunt (2)).

There also have been some indications that in heparinised blood plasma inotropically active peptides could be present in trace amounts (3). Until recently, it has been difficult to isolate trace peptides from blood plasma in sufficient quantity and purity for structural work. However, a technique suitable for this purpose has been worked out in this laboratory and has been presented recently (4) and will be described in detail elsewhere. Using this technique, it has been possible to isolate from blood plasma several inotropically active peptides.

In this contribution we wish to describe one of these peptides which had a particularly striking inotropic activity.

Material and Methods

The peptide was isolated from an intermediate molecular weight fraction (1000 -10000 daltons) obtained by membrane ultrafiltration of heparinised dog blood plasma (4). This was achieved by filtering the plasma through 'Diaflo' membranes (under a constant pressure of 60 psi), which pass molecules with a molecular weight below 10000 daltons (UM-10) and retain molecules with a molecular weight over 1000 daltons (UM-2).

Further purification was carried out by Sephadex gel filtration using Sephadex G-25 and G-10 columns respectively.

The peptide with a molecular weight over 1000 daltons, exhibited an anomalously high adsorbtion on the Sephadex G-25 column, which delayed its elution to a point far beyond the region of low molecular weight components of plasma such as aminoacids and salts. This property, which was described for the first time by Flodin (5) for the heterocyclic ring-containing molecules was used to isolate and purify the peptide using gel filtration at 4°C on Sephadex G-25 (2.5 x 90 cm) and G-10 (2.5 x 30 cm) columns in 0.1N N-Ethylmorpholine buffer pH 7.5 or distilled water.

Aminoacid analysis of a 72-hour mineral acid hydrolysate in 6N-HCl at 110°C, carried out according to Spackman, Stein and Moore (6) revealed only two aminoacids - isoleucine and glycine in the ratio 7:1 and an as yet unidentified organic molecule which gave a positive reaction with ninhydrin.

The study of the aminoacid sequence of the peptide component has been performed by dansyl (7) and dansyl-Edman (8) techniques.

Biological activity was demonstrated using dog heart papillary muscle preparations. The peptide was found to increase the force of a single isometric twitch in dog papillary muscle.

Results and Discussion

Approximately 260 µg of this compound (value deduced from the amount of aminoacids found by the analysis of a total hydrolysate) were obtained from 1 litre of heparinised dog plasma ultrafiltrate.

Ten µg of this compound exhibited an inotropic activity similar to that of 1 µg of adrenaline, or a 0.5m Molar increment of Ca++ in Tyrode solution (1.9mM Ca++). Considering the fact that the molecular weight of this compound is about 10 times higher than that of adrenaline, the inotropic activity based on molecular concentration seems to be very similar to or a bit higher than that of adrenaline. The qualitative difference in action of these two compounds was as follows:

The action of adrenaline was quick and of short duration while the action of the peptide under the same condition was slower and long lasting. This activity was completely destroyed by mineral acid hydrolysis. Chemical analysis (6) of the compound indicated it to be a complex of peptide and an organic molecule "X".

The chemical nature of this compound "X" which was compared with all known aminoacids and their derivatives is still unknown, although it has been established that it is a cyclic compound attached to the N-terminal isoleucine residue. Partial acid hydrolysis in 6N-HCl at 110°C for 20 minutes liberated the peptide component from compound "X".

Structural studies of the peptide component using dansyl (7) and Edman-dansyl techniques (8) showed that the whole molecule could be dansylated, producing only one yellow fluorescent spot when chromatographed two-dimensionally on polyamide thin-layer plates.

Partial mineral acid hydrolysis of the dansylated derivative of the original complex peptide (20 minutes at 110°C in 6N-HCl) followed by thin-layer chromatography of its dried residue resulted in two yellow fluorescent dansyl products (Y1, Y2) and one undansylated fragment

detectable as a brownish (B) spot in ultraviolet light. One of the yellow fluorescent products (Y2) displayed the pattern of the original dansyl peptide so that it was concluded that only two new fragments Y1 and B were obtained from partial acid hydrolysis. Fragment Y1, when subjected to total mineral acid hydrolysis, was destroyed, similarly to the DNS-terminal residue of the intact molecule. It was therefore concluded that fragment Y1 was the DNS "X" compound. When the 18-hour mineral acid hydrolysis of fragment B was redansylated, four DNS-fragments were detected.

Each of these was isolated by two dimensional thin-layer chromatography, extracted into 80% v/v acetone, evaporated and subjected to mineral acid hydrolysis. Although the chromatographic pattern of each intact fragment was distinctly different, the N-terminus was found after hydrolysis in each case to be DNS-isoleucine. The 18-hour mineral acid hydrolysis of the dansyl peptide Y2 after redansylation produced three different dansyl fragments (Y21-Y23), two of them identical to those obtained from fragment B and one different one. All of them were found to have isoleucine as the N-terminal residue.

Free aminoacids in mineral acid hydrolysates of the dansylated fragments detected by thin-layer chromatography and ninhydrin have occurred in individual peptides as follows:

B: isoleucine, glycine; B1: isoleucine and glycine;

B2: isoleucine and glycine; B3: glycine;

B4: isoleucine.

The free aminoacids detected after total hydrolysis of fragments derived from Y2 were as follows:

Y2: isoleucine and glycine; Y21:(identical to B1): isoleucine and glycine;

Y22 (identical to B2): isoleucine and glycine; Y23: isoleucine.

TABLE I

The tentative structure of the fragments of an inotropically active peptide from dog blood plasma.

Fragment	Tentative Structure
B (Whole)	iLeu. (iLeu, iLeu, iLeu, Gly, iLeu, iLeu)
B1	iLeu. (iLeu, iLeu, Gly)
B2	iLeu. (iLeu, Gly)
B3	iLeu. Gly
B4	iLeu. iLeu
Y21	iLeu. (iLeu, iLeu, Gly)
Y22	iLeu. (iLeu, Gly)
Y23	iLeu. (iLeu, iLeu)
A	X. (iLeu, iLeu, iLeu, iLeu, Gly, iLeu, iLeu)

The structure deduced:

X. iLeu. iLeu. iLeu. iLeu. iLeu. Gly. iLeu. iLeu.

A. = intact molecule

B1-B4 = fragments frommineral acid hydrolysis of B

Y21-Y23 = fragments from mineral acid hydrolysis of Y2

According to these results the sequence presented in Table 1 could be deduced.

To corroborate these findings a combined Edman-dansyl technique (8) for stepwise degradation of the peptide was carried out. It was found

that glycine was degraded in the 5th step of the Edman degradation.

The chemical nature of the compound described above is quite unique among the inotropically active peptides isolated so far from dog blood plasma. It is deserving of close study with particular regard to its function in heart failure and it will be the aim of this group to further define the properties of the compound in terms of its clinical significance.

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