Isolation and characterization of variant IGF-1 as well as IGF-2 from adult human brain

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Received 26 March 1986

The forms of somatomedin present in the adult human brain have been characterized in this study. Two peptides were purified by acidification, size exclusion chromatography, affinity chromatography, FPLC and HPLC. Structural analysis identified these peptides as the variant form of IGF-1 with a truncated N-terminal region earlier isolated from human fetal brain and IGF-2. The presence of the truncated IGF-1 variant and IGF-2 in the human CNS suggests their role as neuropeptides.

Somatomedin *Insulin-like growth factor 1* *Insulin-like growth factor 2* *Amino acid sequence* *Central nervous system* *Neuropeptide*

1. INTRODUCTION

The somatomedins or insulin-like growth factors are a family of growth-promoting peptide hormones. Insulin-like growth factors 1 (IGF-1) and 2 (IGF-2) were first purified and characterized from adult human plasma [2,3]. The primary structures of the prohormone forms of IGF-1 and IGF-2 have been deduced from their cDNA sequences [4–7]. Variant forms of both IGF-1 [8] and IGF-2 [9,10] have also been identified. Whilst the somatomedins have been proposed to act as neural growth and maintenance hormones [11], they have yet to be characterized within the mature central nervous system. Although only immunoreactive IGF-2 has been detected in adult human brain extracts and in cerebrospinal fluid [12,13], receptors for both IGF-1 and IGF-2 are found throughout the adult human brain [14,15]. The variant form of IGF-1 with a truncated N-terminal region has been isolated and characterized from human fetal brain [8]. It was unclear however, whether this variant IGF-1 was a specific fetal form or continued to be expressed in the nervous system throughout life. Therefore we have isolated somatomedins from the adult human brain and report here the characterization of both IGF-2 and a truncated IGF-1 variant identical to that found in the fetus.

2. MATERIALS AND METHODS

This study was conducted with ethical committee permission.

2.1. *Purification*

Two whole brains without sign of neuropathology were removed within 9 h post mortem and stored frozen, the tissue was cut into small sections and homogenized in 5 vols of 50 mM Tris-HCl, pH 7.7, containing 0.2 mM PMSF (phenylmethylsulphonyl fluoride) and 0.1 μM pepstatin. The homogenate was centrifuged at 50000 × g for 15 min. Cytosol protein was precipitated by lowering the pH to 5.5 with 1 M acetic acid, centrifugation at 16000 × g for 30 min, further acidification with acetic acid to 0.1 M, incubation overnight at 4°C and centrifugation at 16000 × g for 30 min. The supernatant was concentrated 3 times by rotary evaporation, dialyzed for 20 h with 3 changes of 10 vols of 0.1 M acetic acid with a Spec-

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00145793/86/$3.50

trapor 6 dialysis membrane (Spectrum Medical Industries, USA), further concentrated by rotary evaporation and then chromatographed on a column (3 × 150 cm) of Sephadex G-50 (Pharmacia, Sweden) equilibrated with 0.1 M acetic acid at a flow rate of 24 ml/h. Active fractions were pooled, concentrated, dialyzed overnight against 5 vols of 50 mM Tris-HCl, pH 7.7, and applied to a column (6 × 20 mm) of Sepharose 4B (Pharmacia, Sweden)-coupled amniotic fluid somatomedin carrier protein equilibrated in 50 mM Tris-HCl at pH 7.7. The column was washed with 3 ml of 50 mM Tris-HCl, pH 7.7, and 3 ml H_2O. Activity was eluted with 6 ml of 1 M acetic acid. Active fractions were pooled, concentrated and applied to an FPLC system using an HR 5/5 column of Mono S (Pharmacia, Sweden) equilibrated in 10 mM ammonium acetate, pH 6.5, and 10% acetonitrile. Elution was performed at a flow rate of 1.0 ml/min with a linear gradient of ammonium acetate at pH 6.5 in 10% acetonitrile. Each peak of activity eluted from the FPLC column was then lyophilized, resuspended in 30% acetic acid and applied to an Ultropac TSK ODS 120T column (4.6 × 200 mm, particle size 5 μm) (LKB, Bromma, Sweden). The column was eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min.

2.2. *Amino acid composition analysis*

Approx. 7 pmol of the native peptides were hydrolyzed for 24 h at 110°C with 6 M HCl/0.5% phenol in evacuated tubes. Liberated amino acids were converted to phenylthiocarbamyl derivatives by coupling with phenylisothiocyanate, and the derivatives were identified by reverse-phase HPLC (Waters) on a C_{18} column (Spherisorb S3 ODS 2: 3 μm; Phase Separations, Queensferry, Wales), using an acetonitrile gradient in a sodium phosphate buffer as described [16].

2.3. *Amino acid sequence analysis*

Approx. 35 pmol of native peptide I and 100 pmol of native peptide II were degraded in an Applied Biosystems 470A gas-phase sequencer.

2.4. *Fetal brain radioreceptor assay (RRA-IGF-1)*

This assay was performed as described [17]. Human fetal brain plasma membrane was used as matrix. Purified IGF-1 was iodinated by the lactoperoxidase method and used as ligand. Values were expressed in relation to a human reference serum standard given an arbitrary value of 1 U/ml. The potency of crossreaction in this assay is variant IGF-1, IGF-1, IGF-2 (5:1:0.5) [8].

3. RESULTS AND DISCUSSION

Two forms of somatomedin were purified from the adult human brain according to the scheme given in table 1. The somatomedin activity was monitored by fetal brain RRA-IGF-1 [17]. Cytosol was prepared from brain tissue, acidified and then

Table 1

Purification of fetal brain RRA-IGF-1 from adult human brain

Purification step	Specific activity (U/mg)	Purification	Recovery (%)
Cytosol	0.013	1.0	100
Acid precipitation	0.012	0.9	68
G-50 A-pool	0.040	3.1	27
B-pool	0.090	6.9	11
B-pool			
Affinity chromatography	[a]	[a]	10
FPLC Mono S	[a]	[a]	9
HPLC Ultropac I	30000	2307700	4
II	2900	223100	4

[a] Protein content not determined

chromatographed on a Sephadex G-50 column equilibrated in 0.1 M acetic acid. Fetal brain RRA-IGF-1 activity eluted as two peaks at positions corresponding to apparent molecular masses of approx. 12 and 4 kDa, respectively. The low-molecular-mass active fraction was concentrated, dialyzed against 50 mM Tris-HCl, pH 7.7, and then further purified by means of affinity chromatography on a column of Sepharose-coupled somatomedin carrier protein [18]. The activity was eluted from the affinity column with 1 M acetic acid. The eluate was concentrated and applied to a cation exchange FPLC column equilibrated in 10 mM ammonium acetate, pH 6.5, containing 10% acetonitrile. The elution was performed with a linear gradient of 10–300 mM ammonium acetate, pH 6.5, in 10% acetonitrile. Fig.1A shows the elution profile obtained. Fetal brain RRA-IGF-1 activity eluted as two peaks, denoted I and II, at 180 and 300 mM ammonium acetate, respectively. Peak I contained 75% and peak II 25% of the total activity eluted from the column. After lyophilization, final purification of peaks I and II was obtained by reverse-phase HPLC (fig.1B). Both elution patterns show a single peak of fetal brain RRA-IGF-1 activity at approx. 37% acetonitrile, with peak I eluting slightly before peak II. In this assay, the specific activities of peaks I and II were approx. 30000 and 2900 U/mg protein, respectively. The protein con-

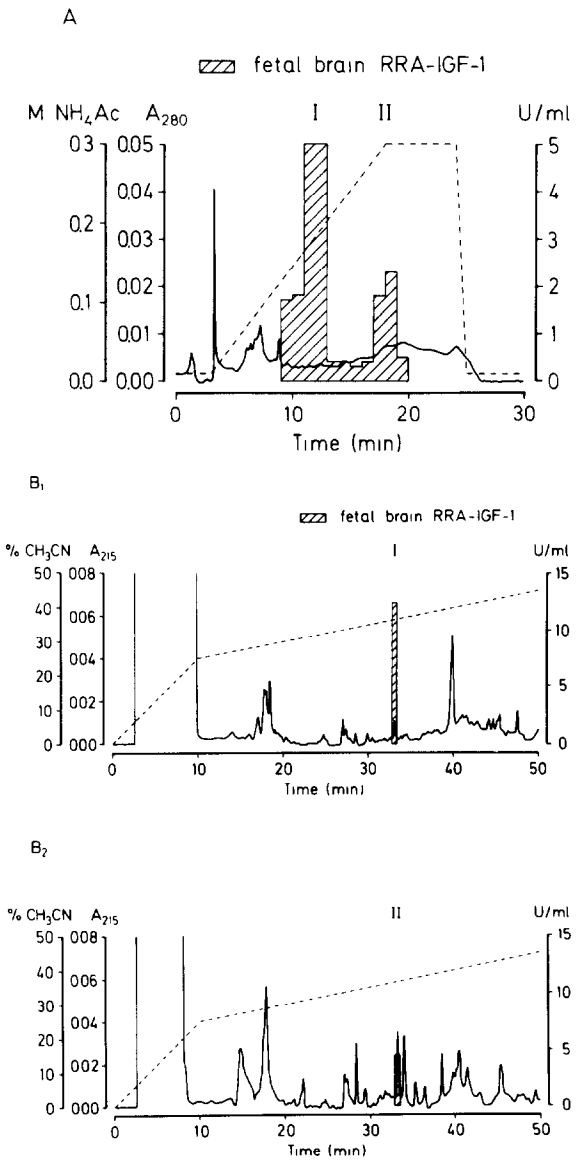

Fig.1. Final steps in the purification of variant IGF-1 and IGF-2 from adult human brain. (A) FPLC separation of the fetal brain RRA-IGF-1 active material. (B) Reverse-phase HPLC purification of peaks I (B_1) and II (B_2) eluted from the FPLC column. Fetal brain RRA-IGF-1 activity is shown by the hatched area.

Table 2

Total compositions of peptides I and II as obtained from analysis of acid hydrolysates, compared to the known compositions of IGF-1 and IGF-2 isolated from human adult plasma [2,3]

Residue	Peptide I	Peptide II	IGF-1	IGF-2
Asx	5.0	4.6	5	3
Glx	5.6	5.2	6	7
Ser	4.8	7.3	5	7
Gly	6.2	4.9	7	5
Thr	2.5	3.9	3	4
Ala	4.7	4.6	6	5
His	0.0	0.1	0	0
Pro	3.7	3.1	5	3
Arg	4.2	8.2	6	8
Tyr	1.8	1.8	3	3
Val	3.4	4.0	3	4
Met	0.5	0.0	1	0
Cys	n.d.	n.d.	6	6
Ile	1.2	0.9	1	1
Leu	4.5	4.7	6	6
Phe	3.0	2.8	4	4
Lys	2.4	1.5	3	1

n.d., not determined

tent was determined by amino acid analysis after acid hydrolysis. The amino acid compositions of peptides I and II isolated from brain as well as those of IGF-1 and IGF-2 isolated from human adult plasma [2,3] are given in table 2. These results show that peptides I and II have amino acid compositions similar to IGF-1 and IGF-2, respectively.

Analysis by gas-phase sequencer degradations revealed peptide I to have a truncated N-terminal region as compared to IGF-1 (fig.2A). The N-terminal threonine of peptide I aligned with position 4 of IGF-1. With this alignment, identical amino acid residues were observed, so that positions 1–19 in peptide I were identical with positions 4–22 of IGF-1. This amino acid sequence is

A

B Domain

Peptide I		T L - G A E L V D A L Q F V - G D R G
1-19		
hIGF-1	G P E T L C G A E L V D A L Q F V C G D R G	
1-22		

B

B Domain

Peptide II	A Y R P S E T L - G G E L V D T L Q F V - G D - - F Y F S - P A	
1-32		
hIGF-2	A Y R P S E T L C G G E L V D T L Q F V C G D R G F Y F S R P A	
1-32		

C Domain

Peptide II	- R V - R R - R	
33-40		
hIGF-2	S R V S R R S R	
33-40		

Fig.2. Analyzed regions of the amino acid sequence of peptide I as compared to the amino acid sequence of IGF-1 (A) [2] as well as peptide II compared to the amino acid sequence of IGF-2 (B) [3].

identical to that of the IGF-1 variant recently identified in human fetal brain [8]. Variant IGF-1 most likely arises from posttranslational modification of pro-IGF-1 since Thr-4 does not correspond to a known intron/exon hinge region [4] and there is no evidence for a second IGF-1 gene. Gas-phase sequencer degradations of peptide II showed the amino acid sequence of this peptide to be identical to the corresponding sequence of IGF-2 (fig.2B). It was possible to identify Ser-29, Ala-32, Val-35 and Arg-38 which demonstrated that brain IGF-2 was not the variant form reported by Jansen et al. [9]. Similarly, the appearance of Val-35, Arg-37 and Arg-38 excluded the possibility that brain IGF-2 was the variant form reported by Zumstein et al. [10]. Thus, IGF-2 in the human brain appears identical to the 7.5 kDa IGF-2 in serum [3].

With this report, variant IGF-1 as well as IGF-2 have now been identified in the fetal and adult brain of man. The truncated variant IGF-1 has so far only been isolated from the central nervous system suggesting the possibility of specific processing within this tissue. In preliminary studies, variant IGF-1 crossreacts weakly with some IGF-1 antibodies, possibly accounting for the lack of IGF-1 immunoreactivity reported earlier in brain extracts [12]. The presence of variant IGF-1 explains the finding of type I receptors in brain tissue [14,15]. These IGF-1 receptors are widely distributed throughout the adult human brain [14]. The identification of variant IGF-1 together with IGF-2 as neuropeptides throughout life will stimulate studies to further elucidate their role in the central nervous system and their involvement in clinical disorders such as Down's syndrome [18,19].

ACKNOWLEDGEMENTS

We wish to thank Professor Kerstin Hall and Dr Guilherme Póvoa for their generous gift of the carrier protein affinity column. These studies have been supported by the Swedish Medical Research Council (6825; 3532; 3529), KabiGen AB, the Sävstaholm Foundation, and the Osterman Foundation.

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