Partial characterisation of a TRH-immunoreactive peptide from rabbit prostate

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A peptide similar to thyrotrophin-releasing hormone (TRH;pGlu-His-ProNH2) has recently been isolated and characterised from rabbit prostate [1] and human semen [2]. This new tripeptide hormone differs from TRH by the substitution of glutamic acid for histidine at position 2. A polypeptide containing 40-50 amino acid residues with a TRH-immunoreactive fragment at its C-terminus has also been detected in rabbit prostate and semen [3] but not in human semen; a larger protein has been detected in human semen [2].

It has been assumed that the C-terminal fragment of the polypeptide in rabbit prostate is identical to or at least related to the novel prostatic peptide pGlu-Glu-ProNH2 which occurs in very high concentrations in male reproductive tissue and secretions but, of course, it is necessary to isolate and characterise the tryptic fragment in order to prove this assumption. Once proven, the N-terminal sequence of the polypeptide can be used to isolate the full-length precursor sequence by cDNA cloning techniques.

The prostate complexes from 18 male rabbits (Sand-lope) were removed and immediately placed on dry ice before storage at -70°C. The tissue was homogenised in acidified acetone to prevent proteolysis of polypeptide, rotary-evaporated to remove the acetone and then redissolved in 25% acetic acid. The sample was loaded on a column of Sephadex G-50 superfine in 25% acetic acid in order to separate the polypeptide from the low molecular weight pGlu-Glu-ProNH2. The position of elution of the polypeptide from the gel exclusion column was determined by TRH radioimmunoassay after tryptic digestion of aliquots of each fraction.

The immunoreactive fractions were pooled, dried, trypsinised and subjected to cation-exchange chromatography at pH2.0 (which separates positively-charged peptides), anion-exchange chromatography pH7.6 (which separates negatively-charged peptides) and then finally purified by reverse phase (C18) high performance liquid chromatography. A portion of the purified tripeptide was hydrolysed for 20h at 110°C in 6M HCl, 2 mM phenol in the vapour phase under N2 and analysis was performed using an Applied Biosystems 420A derivatiser-analysar fitted with an on-line 130A PTC-HPLC detection [4].

The purified tripeptide was neutral both at pH2.0 and 7.6 and remained unbound during both cation- and anion-chromatography. Amino-acid analysis after acid hydrolysis gives the composition 2 Glu16Ser. N-terminal sequence analysis has shown that the peptide is blocked and is likely to contain an N-terminal pyroglutamic acid (thanks to Dr Ian Blench, Imperial College, London). The new peptide does not stand mild acid hydrolysis under conditions which were used to partially uncyclise pyroglutamic acid so that N-terminal sequence analysis could be performed [1,2,5]. From the amounts of amino acids obtained after amino acid analysis it could be deduced that the Glu-Ser peptide cross-reacts approximately 1% with the TRH antibody. In this context, it is interesting that the synthetic peptide pGlu-Gln-Ser NH2 also cross-reacts 1% with the TRH antibody. Further work is in progress to confirm the identity of the new peptide by fast atom bombardment mass spectrometry.

Thus it would appear that the 40-50 residue polypeptide present in rabbit prostate and semen is completely unrelated to pGlu-Glu-Pro NH2 and indeed to TRH itself. It is likely that the new peptide cross-reacts with the TRH antibody by virtue of pyroglutamic acid at position 1. It should be noted that both N-terminal pGlu and C-terminal ProNH2 are necessary for good cross-reactivities with the TRH antibody.

In summary, the polypeptide containing Glu-Ser peptide at its C-terminus must be present in extremely high concentrations. Furthermore, very great caution must be exercised in interpreting TRH immunoassay data.

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