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ISOLATION AND SEQUENCE OF A NON-OPIOID PEPTIDE DERIVED FROM PROENKEPHALIN

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A non-opioid peptide derived from adrenal proenkephalin has been isolated and sequenced. The sequence of this peptide is Ser-Pro-His-Leu-Glu-Asp-Glu-Thr-Lys-Glu-Leu-Gln (Proenkephalin 168-180). This sequence represents the portion of Peptide I that is cleaved to yield Peptide E. This peptide is processed in a similar manner to the opioid peptides and is present at approximately the same level as Peptide E.

Although the sequence of the adrenal proenkephalin has been determined by DNA cloning (1-3) and many of the fragments have been isolated and sequenced (4), the steps involved in processing have not been elucidated. All of the fragments which have been isolated and sequenced to date contain enkephalin sequences and the fate of the non-opioid peptides is unknown. A recent report (5) showed that a peptide corresponding in amino acid composition to the 1-72 sequence of proenkephalin was present in brain. This peptide was missing the enkephalin sequence found at the carboxyterminal end of an 8600 dalton enkephalin-containing polypeptide isolated earlier from the adrenal gland (6). We report here the isolation and characterization of an internal proenkephalin peptide from bovine adrenal chromaffin granules which contains no enkephalin sequence. The peptide represents the 168-180 sequence of proenkephalin and appears to have been processed to a final form in the same manner as the adrenal opioid peptides.

MATERIALS AND METHODS

Chromaffin granules were prepared from fresh bovine adrenal glands as described previously (7). The granules were suspended in 50 mM Tris-HCl (pH

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8.0 at 4°C), sonicated in 30 sec bursts for 2 min and centrifuged at 26,000 x g for 1 hr. The supernatant solution was passed over a soybean trypsin inhibitor-Sepharose column to remove the "trypsin-like enzyme" (7). The flow through from this column was acidified to 1 M acetic acid and β-mercaptoethanol was added to 0.1%. This extract was loaded onto a Sephadex G-100 column (5 x 100 cm) and eluted with 1 M acetic acid, 20 mM HCl containing 0.1% β-mercaptoethanol (8).

The high performance liquid chromatography system (9) was used with pyridine/acetate buffer (pH 4.0) and 1-propanol as the organic modifier. The details of each chromatography are provided in the figure legend. Radioimmunoassays were performed with an antiserum from Immunonuclear (Stillwater, MN) using ^{125}I-Leu-enkephalin (New England Nuclear, Boston, MA) as has been described (10). Fractions were treated with trypsin and carboxypeptidase prior to assay (11).

Amino acid analyses were performed by two different methods. The first was based on the o-pthalaldehyde pre-column labeling system described by Jones, et al. (12). A Spectra Physics 8700 HPLC system and a Schoeffel/Kratos 950 fluorometer were used with an Alltech 3μ C_{18} column (4.6 x 150 mm). The gradient was 50 mM Na Acetate (pH 5.9) to 80% methanol in the same buffer with 1% tetrahydrofuran in both. The other method used for amino acid analysis was the post-column fluorescamine system as has been described (13).

Microsequence analysis was performed on approximately 500 picomoles of the peptide using an Applied Biosystems model 470A protein sequencer similar to that described by Hewick, et al. (14). Phenylthiohydantoin amino acids were identified by HPLC using a Beckman-Altex Ultrasphere ODS column and the trifluoroacetic acid/acetonitrile buffer system reported by Hawke, et al. (15).

RESULTS

Following chromatography of the chromaffin granule extract on the Sephadex G-100 column the fractions corresponding to peak IV (6) were pooled and pumped onto an Alltech 10μ C_{18} column (Figure 1,A). The major peaks which had very

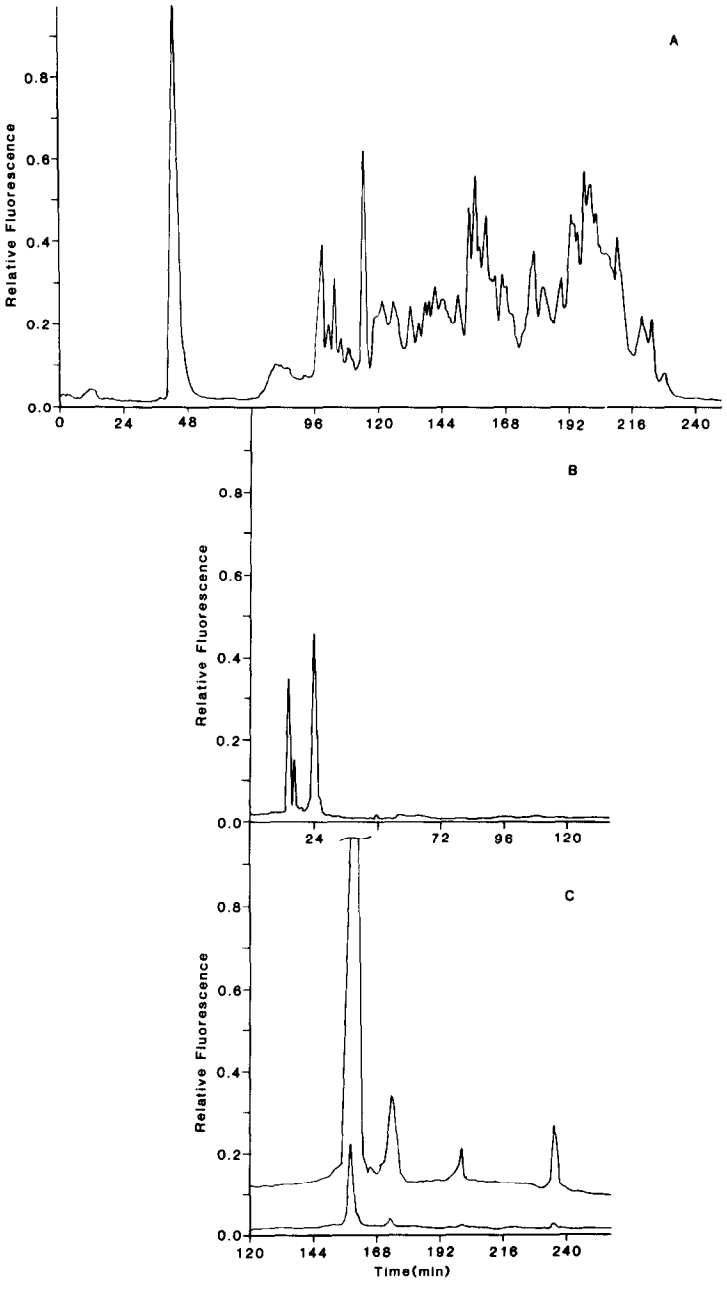

Figure 1. HPLC Isolation. A) Fractions from the Sephadex G-100 column corresponding to molecular weights of 1000-5000 were pooled and pumped directly onto an Alltech 10μ C_{18} column. The peptides were eluted with a gradient of 1-propanol in 0.5 M acetic acid brought to pH 4.0 with pyridine. The gradient was a gradient of 0-18.6% propanol in 140 min, 18.6-29.3% in 60 min and 29.3-40% in 60 min. B) Fractions 23-24 (96-102 min) were pooled and vacuum concentrated. The sample was diluted to 1 mL with starting buffer and injected on a Bakerbond 5μ C_{18} column. The buffers were as in A with a gradient of 6.7-13.3% 1-propanol in 120 min. C) Fractions 8-9 (22-26 min) were pooled and vacuum concentrated. The sample was diluted to 1 mL as in B and injected on a Bakerbond 5μ C_{18} column. The buffers were as in A with a gradient of 0-6.7% 1-propanol in 220 min. Fractions 49-51 (156-162 min) were pooled and used for further studies.

TABLE I

Amino Acid	Precolumn	Postcolumn	Peptide
Asp	1.56	0.82	1
Glu	3.72	3.33	4
Ser	1.40	1.01	1
His	1.00	1.01	1
Gly	0.14	0.28	-
Thr	1.28	0.97	1
Arg	0.48	0.22	-
Ala	0.56	0.19	-
Tyr	0.0	0.07	-
Met	0.46	0.03	-
Val	0.36	0.09	-
Phe	0.0	0.04	-
Ile	0.0	0.03	-
Leu	2.04	2.04	2
Lys	0.84	1.00	1
Pro	N.D.	1.04	1

low or no enkephalin immunoreactivity were pooled and further purified. Fractions 23 and 24 (96-102 min) were vacuum evaporated and chromatographed on two more C_{18} columns (Figure 1, B and C). As seen in the final chromatography a symmetric peak was obtained. The peptide was vacuum evaporated, injected onto a clean C_8 column, and concentrated in a small volume (200 L) by pulsing off the column with 40% 1-propanol. Amino acid analyses and sequencing were carried out on this sample.

Amino acid analyses indicated a small peptide. The two methods of analyses gave similar results (Table I). Sequence analysis gave a sequence of Ser-Pro-His-Leu-Glu-Asp-Glu-Thr-Lys-Glu-Leu-Gln. This sequence corresponds to residues 168-180 of bovine proenkephalin (1,2). This peptide also represents the amino terminal portion of Peptide I (16).

DISCUSSION

There are a number of interesting features observed with this peptide. As seen in Figure 2, at least two "trypsin-like" cleavages are required to liberate this peptide from the proenkephalin precursor. A number of "trypsin-like" activities have been suggested as being responsible for these cleavages (7,17,18). The evidence from this peptide suggests that the enzyme cleaves on the carboxy side of the basic residue since there is no basic amino acid

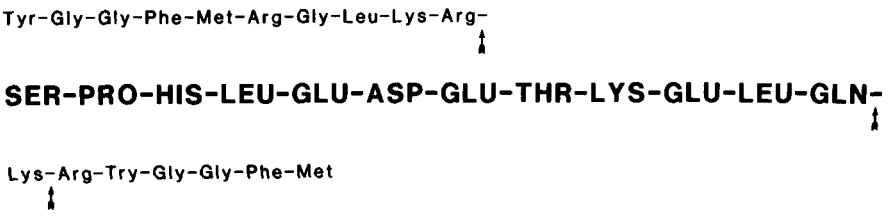

Figure 2. Peptide Sequence. The sequence of the isolated peptide is shown in bold letters in the center. The apparent necessary cleavages are shown by the arrows.

present at the amino terminus. The enzyme also has an apparent specificity for pairs of basic residues since cleavage has not occurred at the internal Lys. In addition, the Lys residue which would be present at the carboxy terminus following cleavage at the paired basic residues has been removed, presumably by a carboxypeptidase (19). This is in apparent contrast to the recently identified proenkephalin peptide from brain. If this peptide is in fact the 1-72 sequence and not 1-70 then in the brain a carboxypeptidase has not removed the basic amino acids at the carboxy terminal.

Whether this peptide represents a non-opioid hormone derived from adrenal proenkephalin is not known at this time. The fact that it is present in substantial quantities in the chromaffin granules, that it has been processed in the same manner as the enkephalin-containing peptides and that there are only two amino acid differences in this peptide between human and bovine (1-3), supports a possible hormonal role for this peptide.

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