

PEPTIDE F (PRO-ENKEPHALIN FRAGMENT):
RADIOIMMUNOASSAY, AND STRESS-INDUCED CHANGES IN ADRENAL

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Summary

Utilizing a nine amino-acid (Asp-Glu-Leu-Tyr-Pro-Leu-Glu-Val-Glu) non-enkephalin containing fragment of Peptide F from the pro-enkephalin molecule, a radioimmunoassay was developed. Extraction of bovine, rat, and guinea pig adrenomedullary preparations demonstrated this fragment to be present and apparently partially conserved across species. In rats, acute inescapable foot-shock stress led to a significant decrease of the immunoreactive material in the adrenal medulla. Chronic daily stress for two weeks resulted in an inability of the adrenals to alter F levels upon subsequent stress. The existence of F-like immunoreactivity and its alteration by environmental manipulation, suggest that it may play a unique physiological role.

A number of findings have led to the rapid isolation and final sequencing of pro-enkephalin in the adrenal medulla (1,2,3). Initially, these findings included the discovery of the presence of enkephalin-like immunoreactivity in the adrenal gland (4,5), and later the isolation of the immunoreactivity to the adrenomedullary chromaffin granules (5,6). Further work revealed a number of enkephalin containing fragments in chromaffin granules characterized by the presence of more than one copy of a biologically active peptide per fragment and their stability after enzymatic degradation (7). Peptide F, a thirty-four amino acid fragment (molecular weight 3840 daltons), was one of the initial sequences of the adrenal medulla pro-enkephalin molecule to be isolated and sequenced.

The question of whether the non-enkephalin portion of the enkephalin precursor possesses any biological function is of importance. The enkephalins are flanked with dibasic residues suggesting trypsin-like cleavages and the possibility of producing novel peptides which could be stored and co-released

with the enkephalins. We therefore developed a radioimmunoassay for the midportion of the F peptide. We have examined the regional distribution of F in the rat brain. We have begun chromatographic studies to characterize the adrenal immunoreactivity. We have also studied physiological changes caused by a stress paradigm, which induces opioid-mediated stress analgesia (8).

The stress paradigm involves administration of acute footshock to the rat to produce naloxone reversible analgesia. When shock is delivered chronically over a period of two weeks "tolerance" appears to develop such that further stress is no longer effective in altering pain. Studies have implicated the adrenal medulla in the opiate-like analgesia seen with the acute stress (9,10).

Methods

Radioimmunoassay

Peptide F fragment. The nine-amino acid sequence was chosen because it did not include the enkephalin structures, had no complex amino-acids and had a tyrosine for iodination. This peptide was synthesized via solid phase method, coupled via glutaraldehyde to thyroglobulin and injected into rabbits. A number of antisera were developed and tested for cross-reactivity prior to their use in the radioimmunoassay. The assay was carried out in potassium phosphate buffer (150mM, pH8.2, 1% NaCl, 3% BSA). After an 18 hr incubation, the charcoal-dextran technique was used for separation of bound from free ^{125}I F peptide.

Tissue Preparation

Bovine adrenals were obtained from a slaughterhouse and stored on ice (15 min after death). The adrenal medullas were dissected out and the chromaffin granules were prepared by a standard procedure with a single modification (12). After isolation the chromaffin granules were homogenized in a solution of 1 M acetic acid, 1 mM HCl for 30 seconds.

Rat and guinea pig adrenal medullas, and rat brains were obtained immediately following the decapitation of the animals. The adrenal medullas were dissected out and frozen on dry ice until the time of further processing. The adrenal medulla from each animal were then homogenized in an acid:acetone solution containing iodoacetamide, and phenylmethylsulfonylfluoride. After the completion of their extraction, tissue specimens were then lyophilized until dry. The rat brains were dissected at the time of decapitation, and sections or regions were processed in the acid:acetone solution (75 vol/wt).

Stress Experiment

Twenty-eight adult male Sprague-Dawley rats were divided into four groups. One group served as non-shocked control. A second group received 30 min of intermittent footshock (5 mA, 1 sec duration, once per 5 sec) immediately before sacrifice (acute group). A third group received the same treatment daily

for 14 days prior to the day of sacrifice. No shock was administered on the last day (chronic group). A final group was identical to the chronic group except the animals were also shocked immediately before decapitation (chronic-acute).

Results

The antiserum chosen showed no cross-reactivity with Met-enkephalin, Leu-enkephalin, ACTH (1-27), alpha-MSH, or Beta-endorphin in our radioimmunoassay. The IC_{50} was 300 fmoles/ml over a number of successive assays. Chromatography of extracts of rat adrenal medulla on Biogel P-2, with 2N acetic acid gave a single peak of immunoreactive material. The peak corresponded to a molecular weight of less than 1500.

Bovine adrenomedullary chromaffin granules, rat and guinea pig adrenal preparations demonstrated the presence of both Leu-enkephalin immunoreactivity and Peptide F fragment immunoreactivity.

The results from the stress experiment are shown in Table I. Our data was analyzed using 4 (conditions) X 3 (trials) repeated measures by Analysis of Variance (ANOVA). The results showed that a statistically significant effect was found from these conditions ($F_{3,24} = 4.76$; $p < .01$). Post hoc analysis using Neuman Keuls indicated that the control group differed significantly from the acute stress group ($P < .05$). In addition, after chronic stress the quantity of Peptide F immunoreactivity was reduced and failed to show a response to acute stress.

TABLE I

Effect of Stress on the Levels of Peptide F
Fragment Immunoreactivity in Adrenal Medullas

Stress Condition	Peptide F Fragment IR (Fmoles/mg wet weight)
Control (n=7)	83.48 \pm 8.42
Acute Stress (n=7)	35.86 \pm 6.34
Chronic Stress (n=7)	52.71 \pm 6.20
Chronic/Acute Stress (n=7)	61.33 \pm 8.00

Values are means \pm SE.

Discussion

The present study demonstrates that an antiserum can be successfully developed which will allow the study of the pro-enkephalin fragments not only in adrenal medulla, but in

brain. The Peptide F fragment appears to be partially conserved across species as demonstrated by its presence in bovine, guinea pig, and rat adrenal medullas, as well as several regions of rat brain. The site of the material suggests that it is further processed beyond cleavage away from the enkephalin. The exact identity of the peptide(s) being measured is under further study. The stress experiment demonstrated that F-like material responds to environmental manipulation. The initial dramatic loss after 30 min is consistent with release of the material from the gland. The lack of further responsiveness to acute stress in the chronically treated animals is consistent with the loss of behavioral analgesia after repeated stress. Taken together, these results suggest a possible physiological role of the F-like material.

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