Neuropeptides 5: 501-504, 1985

ANALYSIS OF OPIOID AND NON-OPIOID END PRODUCTS OF PRO-DYNORPHIN IN THE SUBSTANTIA NIGRA OF THE RAT

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ABSTRACT

The substantia nigra is among the richest pro-dynorphin terminal field regions in the rat brain. We therefore contrasted processing in this area to the known processing in the posterior pituitary. Fractionation of acid extracts of the posterior pituitary by gel filtration followed by analysis by radioimmunoassay indicated that the molar ratio of dynorphin A(1-17) to dynorphin A(1-8) averaged 1:2. The levels of dynorphin A-related end products to α-neo-endorphin and bridge peptide (a 2K nonopioid end product of pro-dynorphin) were approximately equimolar; however, the levels of dynorphin B-sized material were 50% lower than dynorphin A levels. Similar analyses of acid extracts of the substantia nigra also indicated that the levels of dynorphin A, α-neo-endorphin, and bridge peptide were approximately equimolar. In this terminal field the levels of dynorphin B-sized material were approximately 60% lower than dynorphin A. A striking feature of the nigral system was that the molar ratio of dynorphin A(1-17) to dynorphin A(1-8) averaged 1:16. Thus, in the nigra, dynorphin A(1-17) is primarily a biosynthetic intermediate rather than as an end product.

INTRODUCTION

Pro-dynorphin is the common precursor for three opioid peptides: dynorphin A, dynorphin B, and α-neo-endorphin (1). In some tissue, dynorphin A(1-17) can be cleaved to yield dynorphin A(1-8), and α-neo-endorphin can be converted to β-neo-endorphin (2). Finally, these opioid peptides could also potentially be proteolytically cleaved to yield leucine enkephalin. Several recent studies indicate that some of these post-translational processing events occur in the posterior pituitary as well as in different regions of the brain (2,3,4). However, a drawback to studying these processing events by analysis of large brain regions, is the inevitable mixing of cell body and terminal field regions. To avoid this we have focused on the steady state levels of pro-dynorphin-related end products in two clearly defined terminal field regions: the posterior pituitary and the substantia nigra. Furthermore, our understanding of the processing of pro-dynorphin-related opioid peptides in the rodent brain has been greatly aided by the isolation of bridge peptide, a nonopioid end product of pro-dynorphin which represents the 2K polypeptide fragment positioned between α-neo-endorphin and dynorphin A in the seqeunce of porcine pro-dynorphin (Dores, Houghton, Watson and Akil, in preparation).

MATERIALS AND METHODS

For these experiments, adult male Sprague Dawley rats (average wet weight: 250 gm) were sacrificed in groups of 5 to 10 and posterior pituitaries (mean wet weight: 1.6 ±0.1 mg) and substantia nigra (mean wet weight: 10.5 ±0.2 mg) were pooled. Experiments were done in triplicate. The tissues were extracted in 10 volumes of acetone: 0.1 N HCl (3:1) as described previously (5). The extracts were concentrated and redissolved in 500 µl of 10% formic acid buffer and fractionated by gel filtration on a Sephadex G-50 column (1 x 54 cm; Pharmacia) equilibrated in 10% formic acid which contained 100 µg/ml bovine serum albumin. The column was eluted with a flow rate of 1.5 ml/hr and 600 µl fractions were collected. The void volume was marked with bovine serum albumin and the total volume was marked with 2,β-mercaptoethanol. Following gel filtration, aliquots of fractions were concentrated under vacuum (Savant) and analyzed by radioimmunoassay. Recoveries averaged 82%.

Several radioimmunoassays to various end products of pro-dynorphin were used in this study. Radioimmunoassays specific for dynorphin A(1-17), dynorphin A(1-8), dynorphin B, and α-neo-endorphin were done as described by Cone et al. (4). The specificities and sensitivities of these antisera are described in Dores et al. (5). Antiserum against synthetic porcine bridge peptide(1-21) (1) was generated in rabbits and was used at a final dilution of 1:15,000, with an average midpoint of 75 fmol/tube.

In order to assay each column run for all the potential pro-dynorphin-related products, the following fractions were assayed: for dynorphin A(1-17), #42-50; for dynorphin A(1-8), #52-60; for dynorphin B, #48-53; for α-neo-endorphin, #50-55; and for bridge peptide, #42-50.

RESULTS AND DISCUSSION

The results of analyses of extracts of rat posterior pituitary are summarized in Fig. 1 and Table 1. Dynorphin A(1-17)-sized material and dynorphin A(1-8)-sized material represented the major forms of dynorphin A in these extracts. On the average the molar ratio of dynorphin A(1-17) to dynorphin A(1-8) in this terminal field was 1:2. These results are in general agreement with previous studies on this terminal field (2,3,4). As shown in Table 1, the levels of bridge peptide-sized material are approximately equimolar with both dynorphin A and α-neo-endorphin; thus, it appears that this polypeptide is a major nonopioid end product of pro-dynorphin post-translational processing in this terminal field. By contrast, the levels of dynorphin B-sized material were 50% lower than Dynorphin A levels.

Analyses of rat substantia nigra extracts are summarized in Fig. 2 and Table 1. As seen in the posterior pituitary, the levels of dynorphin A, α-neo-endorphin, and bridge peptide were equimolar. A striking feature of the substantia nigra is the low levels of dynorphin A(1-17)-related material (Fig. 2). In this terminal field the major dynorphin A product is dynorphin A(1-8) which is present in a 16 fold higher molar concentration than dynorphin A(1-17). Thus, dynorphin A(1-17) appears to function as a biosynthetic intermediate, rather than an end product. The levels of dynorphin B, while higher than dynorphin A(1-17), are on the average 60 % lower than dynorphin A(1-8) or α-neo-endorphin. A recent study has suggested that the opioid

peptides derived from pro-dynorphin may be converted to leucine enkephalin in the substantia nigra of the rat (6). Collectively, these results would indicate that leucine enkephalin could conceivalby be formed from the cleavage of dynorphin B, but not from dynorphin A or α-neo-endorphin. Thus the production of leucine enkephalin could represent a minor pathway, but not a major pathway in the post-translational processing of pro-dynorphin in the substantia nigra of the rat. Furthermore, the levels of leumorphin (7), the COOH-terminally extended form of dynorphin B(1-13), need to be determined before any definitive statements can be made on the production of leucine enkephalin from pro-dynorphin.

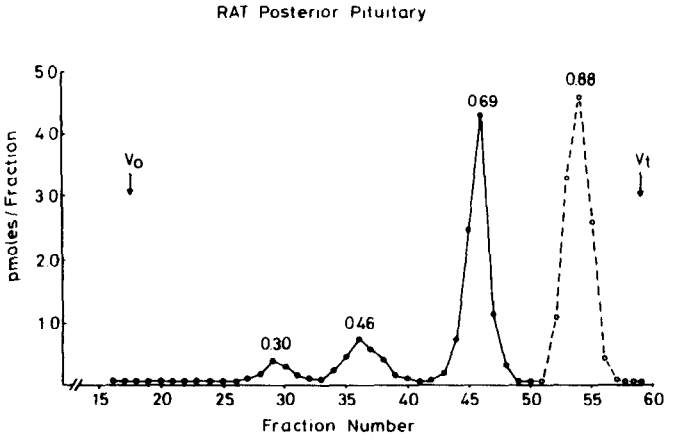

Fig.1. Sephadex G-50 profile of rat posterior pituitary. Ten posterior pituitaries were extracted and chromatographed as described in METHODS. The solid lines represent dynorphin A(1-17)-related material, and the dashed line represents dynorphin A(1-8)-related material.

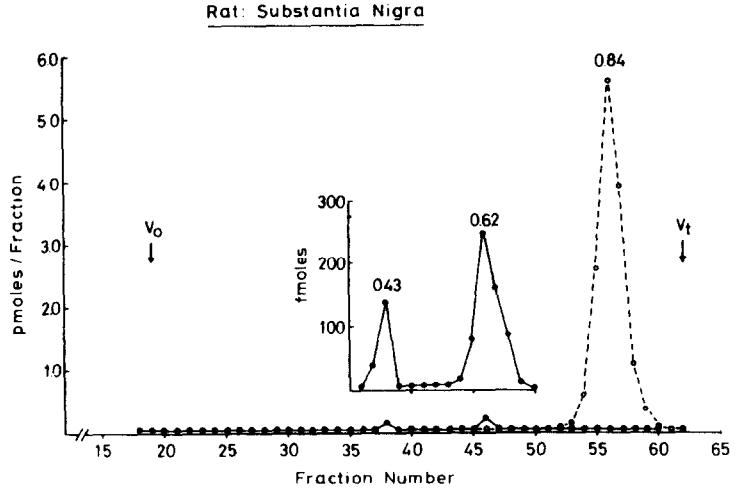

Fig.2. Sephadex G-50 Profile of rat substantia nigra. Five substantia nigra were pooled and extracted as described in METHODS. The solid line represents dynorphin A(1-17) related material, and the dashed line represents dynorphin A(1-8)-related material.

TABLE 1

Peptide	fmol/ mg wet weight Posterior Pituitary	Substantia Nigra
Dynorphin A(1-17)	630 ± 190[1]	20 ± 5
Dynorphin A(1-8)	1190 ± 190	320 ± 20
Dynorphin B	940 ± 380	100 ± 10
α-Neo-endorphin	1250 ± 310	290 ± 30
Bridge Peptide	1440 ± 130	240 ± 20

[1] MEAN ± S.E.M. (n=3)

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