EXTRA HYPOTHALAMO–NEUROHYPOPHYSEAL IMMUNOREACTIVE NEUROPHYSIN OCCURS PREDOMINANTLY AS HIGH M_r FORMS IN THE RAT BRAIN STEM

Christiane SINDING, Maryse CAMIER and Paul COHEN

Université Pierre et Marie Curie, Groupe de Neurobiochimie Cellulaire et Moléculaire, 96 boulevard Raspail, 75006 Paris, France

Received 18 February 1982

1. Introduction

Extra hypothalamo–neurohypophyseal neurophysin-like material has been demonstrated by immunocytochemical methods in various areas of the brain, where it is likely to be associated with ocytocin and vasopressin-like materials [1–3]. These areas can be classified within 2 groups:

(i) Areas probably involved in the classical effect of vasopressin on memory (septum, hippocampus) [4,5];

(ii) Areas and pathways possibly involved in the integration of neuroendocrine autonomic mechanisms (review [6]).

Neurohypophyseal peptides may act as neurotransmitters or neuromodulators in these areas [7]. Numerous peptides initially discovered in the hypothalamus or the pituitary have been located throughout the brain. Questions concerning the biosynthetic pathways of the peptides are a crucial issue.

We have established that in the hypothalamo–neurohypophyseal tract, large forms of neurophysin/vasopressin are present. Besides the 20 000–25 000 M_r species identified in [8–11], a heavier form (M_r 80 000) [12–14] was detected and confirmed [15,16]. This molecule termed coenophorin was proposed as a composite molecule harbouring several brain peptides in its structure. Its shares sequence homologies with neurophysin and adrenocorticotropin (ACTH) [14,17] and bears the antigenic determinants of both vasopressin and β-endorphin [12,13].

The aim of this work was to:

(i) Identify by radioimmunological (RIA) methods the neurophysin-like material throughout the brain;

(ii) Assess the molecular size distribution of immunoreactive neurophysin found in significant amounts outside the hypothalamoneurohypophyseal tract.

2. Methods

Both neurophysin and ACTH radioimmunoassays were used as in [13,18]. The rat neurophysin III and the antibody were kindly given by Dr A. G. Robinson. To rule out RIA artefacts, the samples were assayed with and without preincubation with ^{125}I-neurophysin and the results found to be similar. When the immunoreactive neurophysin from the brain stem was compared to the rat reference neurophysin over a 20-fold range, it was found to behave similarly. All other techniques and controls are described in the legends of table 1 and fig.1.

3. Results and discussion

The concentration of immunoreactive neurophysin was surprisingly low in the areas which are supposed to be involved in the memory process (table 1) and the highest concentration was found in the brain stem (close to 1/40-fold the concentration in the hypothalamus). This result is consistent with histological data which demonstrated the retrograde-transport of horse radish peroxidase from the spinal cord and the brain stem to the hypothalamus [19]. Immunohistochemical data have established the existence of neurophysin [1,2,20] in vasopressin and ocytocin fibers [21] which arise from the hypothalamus (mainly the paraventricular nucleus) and project to the brain stem and

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

the spinal cord, in the preganglionic parasympathetic nucleus as well as sympathetic cell groups (intermediolateral column).

Extracts made from rat brain stems were subjected to gel filtration on a Sepharose CL-4B column. Drastic conditions of gel filtration (performed in the presence of 6 M guanidine) were used in order to prevent from the aggregation of small molecules. The RIA of neurophysin-like material in the collected fractions (fig.1A) revealed a pattern which was strikingly different from the now well-characterized pattern obtained in the hypothalamo–neurohypophyseal tract [10,22]: >90% of the immunoreactive species were >10 000 M_r neurophysin; ~20% behaved as molecular forms ≳20 000 M_r.

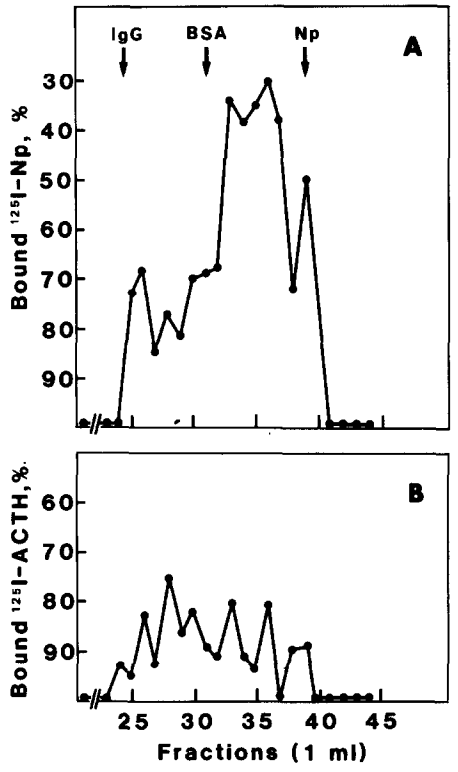

Fig.1. Patterns of elution of rat brain stem extracts after gels filtration on a column (300 × 9 mm) of Sepharose CL-4B. Brain stem tissues were extracted in 0.1 M HCl, centrifuged at 100 000 × g then 6 M guanidine was added to the supernatant. Gel filtration was conducted in the presence of 6 M guanidine in 0.1 M HCOOH, 0.1 M NaCl. The eluted fractions were dialyzed against 0.1 M Tris (pH 7.5) for 24 h at 4°C, then lyophylized and redissolved in 1 ml 0.1 M Tris (pH 7.5) buffer. They were assayed both for neurophysin (Np) and ACTH immunoreactivities. (A) Pattern of Np immunoreactivity. (B) Pattern of ACTH immunoreactivity. The arrows indicate the elution peaks of IgG (140 000 M_r) bovine serum albumin (BSA, 68 000 M_r) and ^{125}I-Np (10 000 M_r) added as internal markers. Np and ACTH immunoreactivities are expressed as percentage of ^{125}I-tracer bound to the respective antibodies.

Immunoreactive ACTH has been suggested in the brain stem of several species by immunocytochemistry [23,24] or RIA [25] but nothing is known about its molecular form in this particular region. When assayed for ACTH, the eluted fractions were found to exhibit immunoreactivity for the ACTH antibody. The pattern of fig.1B suggests the presence in the brain stem of high M_r forms of adrenocorticotropin. To which extent the immunoreactive material detected in the elution volume of the largest species (between the IgG and BSA) is related to the coenophorin or to other precursor forms remains to be determined. These data indicate that neurophysin is present in the brain stem predominantly as high M_r species, a situation completely opposite from the pattern observed in the bovine and mouse neurohypophyseal tract where >90% of the immunoreactive neurophysin is present as a ~10 000 M_r form. This observation also suggests that the evaluation of neurophysin in the brain stem as 1/40 of the concentration found in the hypothalamus (table 1) is a lower limit since it can be expected that high M_r forms exhibit a significantly lower immunoreactivity than the free 10 000 M_r neurophysin.

Although it cannot be strictly stated that we are dealing with the same molecules as the neurohypophy-

Table 1
Concentrations of immunoreactive neurophysin-like material in 6 areas of the brain

Areas	Neurophysin (fmol/mg fresh tissue) (mean ± SEM)
Brain stem	46 ± 13
Nucleus caudatus	15 ± 4
Cerebellum	1.2 ± 0.3
Hypothalamus	1810
Hippocampus	1.6 ± 0.2
Septum	11 ± 0.3

The rats were killed by rapid decapitation, the brain quickly removed and dissected on ice; the tissues were extracted in 0.1 M Tris (pH 7.5)/aprotinin 500 KIU/ml. The concentration of immunoreactive material was determined on 6 expt by RIA [18] and expressed as fmol neurophysin/mg fresh tissue. The number corresponding to the hypothalamus is the mean value of 2 expt.

seal coenophorin [13] and the 20 000–25 000 M_r precursor forms [8,11], it can be inferred that the processing which liberates the biologically active peptides appears to occur differently depending upon the areas where the relevant neuronal cells project their terminals.

Therefore, comparison of the above data with the situation in the neurohypophysis, supports the hypothesis of a differential post-translational processing of the prohormone. Such a mechanism seems to apply to the proopiomelanocortin processed differently in the pars intermedia or the adenohypophysis [26]. Further studies of the biosynthetic pathways of these neuropeptides in various areas of the brain, or outside the brain will shed a new light on the hormone–neuropeptide–neurotransmitter issue.

Acknowledgements

This work was supported in part by funds from the Université Pierre et Marie Curie, the Centre National de la Recherche Scientifique (Equipe de Recherches Associée no. 693, Action Spécifique Programmée and Programme Interdisciplinaire de Recherches sur les Bases Scientifiques des Médicaments), the Délégation Générale pour la Recherche Scientifique et Technique (contracts no. 79-7-0788 and 81-E-0396) and the Fondation pour la Recherche Médicale Française. The guidance of Dr Hamon (Collège de France) in the dissection of rat brain was greatly appreciated.

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