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IDENTIFICATION OF IMMUNOREACTIVE NEUROPHYSIN-LIKE PROTEINS IN THE CENTRAL NERVOUS SYSTEM OF AN INSECT ; *LOCUSTA MIGRATORIA*.

M. Camier[+], J. Girardie[⊕], C. Remy[⊕], A. Girardie[⊕] and P. Cohen[+].

[+]Groupe de Neurobiochimie Cellulaire et Moléculaire
Université Pierre et Marie Curie,
96 boulevard Raspail, 75006 Paris, France
and
[⊕]Laboratoire de Neuroendocrinologie,
Université Bordeaux I, 33405 Talence, France.

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SUMMARY : Proteins exhibiting the immunoreactivity of vertebrate neurophysin were detected in acido-ethanolic extracts of sub-oesophagial ganglia of the locust. These components possess an apparent molecular weight approximating 10,000, acidic isoelectric points (4.5 and 4.75) and bind to antibovine neurophysin antibodies immobilized on CH-Sepharose 4B. These results suggest that the immunoreactive material observed in the *Locusta Migratoria* is composed of proteins structurally related to vertebrate neurophysin.

Neurophysins, low molecular weight (10,000) acidic proteins with a high disulfide content (for reviews see (1-4)),have been detected in a number of vertebrates both in the neurosecretory system and in extra-hypothalamic regions (1-5). High molecular weight forms, putative biosynthetic precursors of these proteins (6-10), together with those of the associated neurohormones, vasopressin (11) and possibly ocytocin, seem to be processed and transferred to the neurohypophysis by axonal transport via neurosecretory vesicles. The neurophysins appear to play a stabilizing role within the granule by ensuring a tight binding of the hormonal nonapeptide ligands and possibly by forming dimeric complexes (4). In only a few examples,vertebrates hormone -like immunoreactivities were detected in the central nervous system of invertebrates (12-20). On the basis of preliminary immunohistological observations (18) we have investigated the possibility that the neurophysin-like reactivity detected in the central nervous system of the migratory locust might correspond to components exhi-

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biting biochemical analogies with the well-described vertebrate neurophysins (1-4). In the present report we provide evidence indicating that a protein material with an apparent molecular weight of approximately 10,000 and a characteristic acidic nature (pH_i 4.5 and 4.75), which is recognized by two different antineurophysin antisera both by radioimmunoassay and affinity chromatography, is detected in the acido-alcoholic extracts of sub-oesophagial ganglia of the insect *Locusta migratoria*.

EXPERIMENTAL PROCEDURE

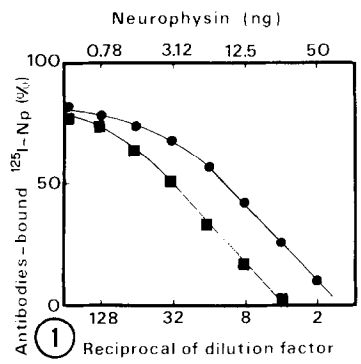
In a typical experiment, 500 sub-oesophagial ganglia were dissected from a population of either male or female gregarious locusts and immediately kept in acetone at 4°. All the operations were run at this temperature. These ganglia, partly delipidified, were vacuum dried and then homogenized with a Potter-Elvejhem in the mixture (0.1N formic acid, 70% ethanol, 5mM phenylmethyl sulfonyl fluoride (PMSF from Sigma, St Louis, Miss.)).

After 16 hours, the homogenate was centrifuged at 11,000g for 15 mn and the operation was repeated on the pellet. Both supernatants were collected and lyophilized. Soluble components were taken up in 500 mM Tris-HCl, pH 7.5 buffer containing Trasylol, 500 kallikrein inhibitor units (KIU) per ml. The neurophysin-like material was detected using the radioimmunoassay (RIA) in the presence of protease inhibitors as previously described (8) and with the A_5IV antiserum supplied by Dr Legros (Liège). Isoelectric focusing of the neurophysin-like material was carried out as previously described (23).

RESULTS AND DISCUSSION

The displacement curve obtained by serial dilutions of the acido-ethanolic extract of sub-oesophagial ganglia is shown in figure 1. Comparison with the reference curve, produced under the same conditions by bovine neurophysin II, indicates that the extract contains ethanol soluble components which compete with the ^{125}I-bovine neurophysin II tracer for binding to the antibodies. Nanograms equivalents of bovine neurophysin-like material were estimated per mg of soluble proteins of the extract (measured by the Folin method).

The molecular weight of this immunoreactive material was then evaluated by molecular sieve filtration. The distribution pattern of the neurophysin-like immunoreactivity in the eluate of the Biogel P-10 filtration of the extract indicates the presence of a major component with the same elution volume as the ^{125}I-bovine neurophysin II standard (figure 2). Besides the



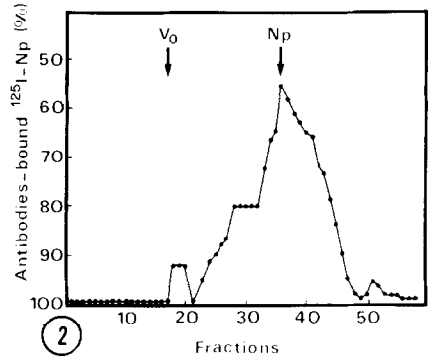


Figure 1 : Radioimmunological analysis of the neurophysin-like components in the extract of locust ganglia.
(●) serial dilutions of the extract analyzed by radioimmunoassay with antibovine neurophysins and ^{125}I-bovine neurophysin II (^{125}I-Np) as tracer.
(■) Reference curve obtained with standard bovine neurophysin II.

Figure 2 : Molecular sieve fractionation of the neurophysin-like components in the extract of locust ganglia. The lyophilized extract was dissolved in 500 mM Tris-HCl, 500 KIU/ml trasylol pH 7.5 buffer and applied to a column of Biogel P-10 (19x1.5cm) equilibrated in 0.1N HCOOH containing 0.1% Triton X-100, 0.5mM PMSF, 1 mg/l pepstatin . The neurophysin-like immunoreactivity was evaluated on each 0.8 ml fraction by RIA. The arrow indicates the elution volume of ^{125}I-bovine neurophysin used as standard. The exclusion volume (Vo) corresponds to material $\geq$ 20,000 daltons. Np : neurophysin.

10,000 daltons components, the presence of higher molecular weight immunoreactive forms was noted both before the elution volume of neurophysin and in the exclusion volume (MW $\geq$ 20,000) of the column. This pattern should be compared with the qualitatively similar ones obtained with mouse hypothalamic (8) or bovine neurohypophyseal preparations (21-22). These high molecular weight forms were taken as possibly representing the biosynthetic putative precursors synthesized on the ribosomes and which may generate neurophysin by post-translational processing (22).

The acidic nature of the neurophysin-like material recovered in the 10,000 daltons elution volume of the column was revealed by isoelectric focusing. The immunoreactive components were found to focus as two identical peaks at pH_i 4.5 and 4.75. These values are closely related to the pH_i of other vertebrate neurophysins, bovine : 4.3 and 4.7 (23), mouse : 4.5 (unpublished results) and rat : 4.8 (24). Finally, a direct demonstration that the 10,000 daltons immunoreactive material can bind anti-bovine neurophysin II antibodies was provided by affinity chromatography on ammonium

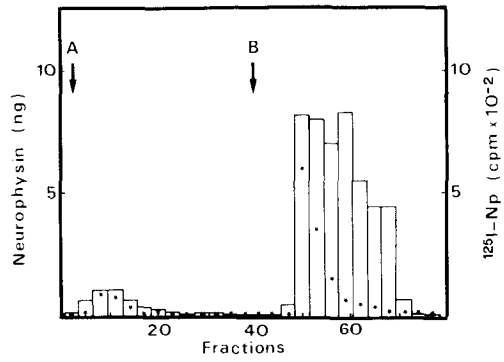


Figure 3 : Affinity chromatography on anti-bovine neurophysin II antibodies immobilized on CH-Sepharose 4B of the 10,000 daltons material from Figure 2 lyophilized and then dissolved in 100 mM phosphate buffer pH 7.5 containing 500 KIU/ml trasylol. The sample was applied on the column (3 ml) in 100 mM phosphate buffer, 100 mM NaCl, pH 7.5. The complex was washed (A) with the same buffer and desorption was obtained (B) with 1M acetic acid. Each fraction (0.3 ml) was lyophilized then tested by RIA. Immunoreactivity is expressed as neurophysin equivalents calculated from the neurophysin standard curve from figure 1. The elution behaviour of ^{125}I-bovine neurophysin (^{125}I-Np) used as standard is represented (★).

sulfate-precipitated anti-bovine neurophysin II antiserum ($SIIO_3$ from this laboratory) immobilized on CH-Sepharose 4B. More than 95% of the material recovered from the Biogel P-10 filtration was found to be adsorbed, as well as the marker ^{125}I-labeled bovine neurophysin, on the immunoadsorbant (figure 3).

This set of observations clearly demonstrates, in the extracts of ganglia, the existence of proteins possessing a molecular weight, isoelectric points and immunochemical properties analogous to those of neurophysin. To the best of our knowledge this is the first example indicating chemical analogies between hormonal components of vertebrate and invertebrate. The biological significance of the neurophysin in insect central nervous system is not yet clear. In both the mouse (11) and ox (22) recent observations suggest that the neurosecretory components, as early hypothesized (25), might be synthesized as part of the same high molecular weight forms, putative precursors of both neurophysin and nonapeptide hormones. If this also occurs in the nervous system of insects, the presence of associated vasopressin could be expected. Preliminary observations indicate the presence in the same neurons of a material which is related both immunologically (18-20) and pharmacologically (unpublished results) to vasopressin. This

would imply that regulatory mechanisms mediated by vertebrate-like components may also exist in such types of insect.

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