Proteolytic Conversion of Arginine-Vasopressin and Oxytocin by Brain Synaptic Membranes

CHARACTERIZATION OF FORMED PEPTIDES AND MECHANISMS OF PROTEOLYSIS*

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This study concerned the fragmentation of the nonapeptides arginine-vasopressin (AVP-(1-9)) and oxytocin (OXT-(1-9)) by proteolytic enzymes present in a brain synaptic membrane preparation. The peptides formed during digestion of arginine-vasopressin and oxytocin were isolated by high pressure liquid chromatography and chemically characterized by amino acid composition, NH₂-terminal amino acid residues, and the presence of ¹³C radioactivity in tyrosine-2 and glycineamide-9. The major peptide fragments of arginine-vasopressin were [Cyt¹]-AVP-(2-9), [Cyt¹]-AVP-(3-9), [Glu¹,Cyt²]-AVP-(4-9), and a peptide having the AVP-(4-8) sequence. The characterized fragments of oxytocin were [Cyt¹]-OXT-(2-9), [Cyt¹]-OXT-(3-9), [Cyt²]-OXT-(4-9), [Glu¹,Cyt²]-OXT-(4-9), and [Cyt²]-OXT-(5-9). Employing differentially ¹³C-labeled arginine-vasopressin and oxytocin, the proteolysis of the two peptides into fragments was followed with time. The results showed the sequential formation of peptide fragments by proteolytic cleavage from the NH₂ terminal onward, demonstrating the action of an aminopeptidase-like enzyme. Arginine-vasopressin was converted significantly more rapidly by the aminopeptidase activity than oxytocin. In contrast to known brain aminopeptidases, the synaptic membrane-associated activity cleaved the nonapeptides without prior reduction of the disulfide bridge. From the present data it is concluded that aminopeptidases predominate in the proteolytic mechanism by which brain synaptic membranes convert arginine-vasopressin and oxytocin. The role of the proteolytic events and the significance of formed peptide fragments is discussed in view of the concept that arginine-vasopressin and oxytocin are precursors for neuropeptides in brain.

The nonapeptides oxytocin and vasopressin have an important role as regulatory peptides for peripheral as well as central functions. Oxytocin and vasopressin are synthesized in neuronal cell bodies in the hypothalamus and transported via axonal fiber systems to the neurohypophysis as well as to limbic and midbrain regions (1-3). In the terminal areas of vasopressinergic and oxytocinergic pathways, the peptides are stored in synaptic structures (4, 5) from which they can be released (6, 7). Oxytocin and vasopressin secreted from the neurohypophysis circulate peripherally and act as hormones serving endocrine integration. In brain, oxytocin and vasopressin are potent neuropeptides affecting a variety of brain functions. The peptides have a role in memory processes (8, 9), neuroendocrine (10, 11) and cardiovascular regulation (12), control of body temperature (7) and brain development (13), induction of maternal behavior (14), and development of tolerance and addiction to narcotic drugs (15, 16).

A clear distinction between the structural requirements for peripheral endocrine activity and for central activity of oxytocin and vasopressin exists. Whereas the complete nonapeptide structure of oxytocin and vasopressin is required for full endocrine activity (17-19), the central activities of the peptides are not limited to the nonapeptide moieties as such. Several fragments of vasopressin and oxytocin have strong central activities, which are different from or independent of those of the parent molecules (20-22). Based on these observations, the neurohypophysial hormones have been indicated as precursors for centrally acting oligopeptides (20, 21, 23).

The postulated precursor function of vasopressin and oxytocin implicates that proteolytic fragmentation of the neuropeptides is a prerequisite for liberation of the smaller neuropeptides (24). Since oxytocin and vasopressin are the stored forms of neurohypophysial peptides, we have considered functional proteolytic processes in brain to take place after secretion of the nonapeptides, e.g. during exposure to the synaptic environment, in analogy to the proposed formation of behaviorally active β-endorphin fragments (25, 26). In previous studies, we have investigated oxytocin converting proteolytic activities present in brain synaptic membrane preparations of rat brain (27) and have tentatively characterized an oxytocin fragment generated by brain proteolysis (28). In the present paper, we have aimed to delineate the mechanisms of the proteolysis of arginine-vasopressin and oxytocin by brain synaptic membranes via the characterization of the peptides which are formed during proteolysis and via the examination of the course of proteolytic events.

MATERIALS AND METHODS

Arginine-vasopressin, oxytocin, and related peptides were synthesized by Drs. H. M. Greven and J. W. van Nispen (Organon International, Oss, The Netherlands). The radiolabeled nonapeptides [¹³C]Tyr⁴-vasopressin, [¹³C]GlyNH₂⁷-vasopressin, [¹⁴C]Tyr⁷-arginine vasopressin, and [¹³C]GlyNH₂⁷-arginine vasopressin were generous gifts of the late Dr. R. Walter (University of Illinois, Chicago, IL) and Dr. W. H. Simmons (Loyola University, Maywood, IL). All radiolabeled peptides were purified before use by high pressure liquid chromatography to a purity of approximately 98% as described under "High Pressure Liquid Chromatography."

Pyroglutamate-aminopeptidase (L-pyroglutamyl-peptide hydrolase, EC 3.4.11.8) from calf liver was obtained from Sigma.

Preparation of Brain Synaptic Membranes—Membranes were prepared from midbrain and forebrain tissue of male Wistar rats, weighing 170-200 g, following a previously described fractionation
subjected to liquid scintillation counting. Fractions from preparative runs with 20% B over 10 min. Membrane protein concentration, sodium acetate, pH 4.15 (solvent A), and methanol containing 1.5% to 40% B in 25 min was used and for vasopressin digests a parallel incubation with ['4C]labeled peptides (10,000–40,000 dpm) was carried out on short linear gradients: for oxytocin preparations, the final concentration of 2×10⁵ M hydrogen chloride for 2.5 h and was terminated by the addition of 1.0 ml of 10 M, were distilled water and 10 mM disodium EDTA under a N₂ atmosphere. Digestion was started by adding 20 μl of pyroglutamate-aminopeptidase in buffer (2 mg of protein/ml) to 5 μl of peptide in buffer (2–5 nmol). After a 4-h incubation at 4°C, a second aliquot of a 20-μl enzyme solution was added and incubation was continued for 16 h at room temperature. In every experiment, the activity of the enzyme was tested on synthetic [Glu'4,Cys'6]-OXT-(4-9) or [Glu'4,Cys'6]- AVP-(4-8).

Performic Acid Oxidation, Acid Hydrolysis, and Determination of Amino Acid Composition—A modification of the method of Hirs (35, 36) was used for performic acid oxidation of peptides. Performic acid was prepared by mixing 1 volume of 30% hydrogen peroxide and 19 volumes of 99% formic acid. The mixture was kept at room temperature for 2 h in a closed vessel and subsequently cooled in an ice water bath. Performic acid (50 μl) was added to the peptide material (0.5–2 nmol), which was dissolved in 25 μl of 99% formic acid and 5 μl of methanol and cooled to 0°C. The reaction was carried out at 0°C for 2.5 h and was terminated by the addition of 1.0 ml of distilled water (0°C). The samples were lyophilized before acid hydrolysis.

Hydrolysis of peptides (0.5–3 nmol) in 6 M hydrochloric acid containing 0.1% thioglycolic acid was carried out as described previously (23). Aliquots of 50–200 pmol were subjected to amino acid analysis. The loss of tyrosine residues under the conditions of peroxidase and acid hydrolysis was minimal. Amino acid analysis was performed by a technique based on reversed-phase HPLC of amino acids which had been pre-column derivatized with o-phthalaldehydide/2-mercaptoethanol reagent (37–40). Amino acid derivatives were prepared in a standardized procedure and separated on a Nucleosil 5C18 column (250 × 4.6 mm inside diameter, particle size, 5 μm, Chrompack, Middleburg, The Netherlands) using gradient elution with solvent C (0.1 M sodium citrate, pH 6.5, methanol (4:1, v/v)) and solvent D (methanol). Elution was monitored by UV absorbance at 210 nm and 1.0-nl (0.5 min) fractions were collected by a 2112 Reidar fraction collector (LKB). Fractions from samples containing ['4C]-labeled peptides were subjected to liquid scintillation counting. Fractions from preparative oxidations were pooled from the UV absorbance profile or the ¹⁴C radioactivity profile of parallel incubations. Membranes were removed from pooled fractions in a Vortex-evaporator (Searle, Fort Lee) at 60°C, lyophilized, and stored for further analyses. Rechromatography of several pooled fractions was carried out by isocratic elution under conditions as described under "Results."
started with a linear gradient from 0% to 30% D in 5 min, followed by
an isocratic step at 30% D for 5 min and a linear gradient to 65% D in
20 min. The procedure was reproducible (average relative deviation
was 3.3%), yielded linear fluorescence responses of amino acids, and
had a detection limit of approximately 5 pmol. Details of the tech-
nique have been described elsewhere (40).

RESULTS

High Pressure Liquid Chromatography of Digests and
Chemical Characterization of Isolated Peptides

Arginine-Vasopressin—During incubation of arginine-vasopressin with isolated brain synaptic membranes for 3 h, a
number of peptide fragments accumulated as was seen after
HPLC fractionation of the digests (Fig. 2). UV monitoring of the
HPLC eluate showed the presence of a component co-
migrating with intact arginine-vasopressin (AVP-I; Fig. 2A)
and four metabolites (AVP-II, -III, -IV, and -V; Fig. 2A). HPLC fractionation of [14C]GlyNH2-vasopressin digests demonstrated that [14C]glycinamide was contained in fractions AVP-I, -II, -III, and -V, as well as in components eluting with earlier retention times (Fig. 2B). [14C]Glycinamide was absent in fraction AVP-IV. Free glycinamide eluted in the void volume. HPLC of [14C]Tyr-vasopressin digests showed that [14C]tyrosine radioactivity was contained in fractions AVP-I and AVP-II and in the component co-eluting with free
tyrosine (Fig. 2C).

**FIG. 2.** HPLC profiles of arginine-vasopressin digests. Vasopressin, 5 X 10^-5 M, was incubated with a brain synaptic membrane preparation for 3 h, with or without addition of the peptide [14C]-labeled in the glycinamide or tyrosine position. After termination of the enzyme reaction, the membrane-free supernatant was subjected to HPLC fractionation on a Bondapak C18 reversed-phase column and eluted with a concave gradient of methanol and ammonium acetate buffer, pH 4.15, over 30 min. The column effluent was monitored by UV absorbance at 210 nm (A). Collected 0.5-min fractions were counted for [14C]glycinamide (B) or [14C]tyrosine content (C). Fractions I, II, III, IV, and V were collected for chemical analyses. The symbols indicated in B (○, +, △, □) and C (○, △, ◆) define the
14C-labeled components which are presented with identical symbols in Fig. 4.

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<th>Amino acid residue</th>
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<td>AVP-(4-9)</td>
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</table>

"Further analyses are described in the text and in Table II.

"b.d., below detection.

"Proline is not detected by the amino acid analysis technique.

"<Glu-AP, pyroglutamate aminopeptidase (EC 3.4.11.8).

n.t., not tested.
For chemical analyses, fractions were collected from preparative incubations of arginine-vasopressin with synaptic membrane material. The isolated fractions were subjected to amino acid analysis after performic acid oxidation and to determination of the free NH₂-terminal residues. Since the amino acid sequence of the digested substrate was known, the identity of the isolated fragments could be established. Moreover, additional structural information was provided by the presence or absence of [¹⁴C]tyrosine or [¹⁴C]glycinamide radioactivity in the isolated fractions. The data are presented in Table I.

Five fractions isolated from vasopressin digests were analyzed. Fraction AVP-I was identified as intact vasopressin (AVP-(1-9)) based on the amino acid composition, the monodansyl-cystine and o-dansyl-tyrosine residues, presence of both [¹⁴C]tyrosine and [¹⁴C]glycinamide (Fig. 2), and co-migration with intact vasopressin. Fraction AVP-II has a similar amino acid composition and contained both radioactive amino acid residues. However, the peptide contained two free NH₂-terminal groups as demonstrated by the presence of monodansyl-cystine and didansyl-tyrosine upon dansylation of fraction AVP-II. It was concluded that fraction AVP-II constituted the open nonapeptide Tyr<sup>1</sup>-Phe-Gln-Asn-Cys<sup>6</sup>-Pro-Arg-Gly<sup>10</sup>NH₂. The peptide in fraction AVP-III was characterized as Phe<sup>1</sup>-Gln-Asn-Cys<sup>6</sup>-Pro-Arg-Gly<sup>10</sup>NH₂.

Fraction AVP-IV was devoid of [¹⁴C]tyrosine and [¹⁴C]glycinamide radioactivity. The amino acid composition of this fraction showed that in a molar ratio one cysteic acid residue was present, while a noncorrelating amount of glycine was found (Table I). The absence of [¹⁴C]glycinamide, however, demonstrated that this glycine did not originate from vasopressin, but was a contaminant in fraction AVP-IV. No major dansyl end group was found in fraction AVP-IV. After treatment with pyroglutamate-aminopeptidase (EC 3.4.11.8) and dansylation, a single dansyl-aspartic acid residue was detected demonstrating the presence of a pyroglutamic acid residue in the peptide. It was concluded that the peptide contained the AVP-(4-8) sequence. Upon HPLC fraction, AVP-IV differed in retention time from [<Glul,Cys<sup>6</sup>]-AVP-(4-8), [Cys<sup>6</sup>]-AVP-(4-8), [<Glul,Cyst<sup>6</sup>]-AVP-(4-8), [<Glul,Cys<sup>6</sup>]-AVP-(4-8) -dimer, as well as from the reduced forms of these peptides as shown in Table II. After reduction with mercaptoethanol, AVP-IV shifted to a longer retention time (Table II). The amino acid composition and HPLC analysis may indicate that fraction AVP-IV constituted a derivatized monomeric form of the AVP-(4-8) sequence, probably oxidized in residue cysteine<sup>6</sup>, or an AVP-(4-8) peptide dimerized with synaptic plasma membrane component. The present analyses did not establish the structure conclusively.

Fraction AVP-V contained a [¹⁴C]glycinamide residue and the amino acid composition indicated the [Cys<sup>6</sup>]-AVP-(4-9) sequence. In addition, a pyroglutamic acid residue was de-
Table III

Analysis of HPLC fractions of oxytocin digests

The values in parentheses are the theoretical numbers of residues based on the composition of the proposed sequence. The data are averages of 2-3 determinations.

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<tr>
<th>Amino acid residue</th>
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<th>III</th>
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<th>V</th>
<th>VIa</th>
<th>VIIb</th>
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* The fraction was rechromatographed by HPLC under isocratic condition (see text) prior to analysis.
* b.d., below detection.
* Proline is not detected by the amino acid analysis technique.
* <Glu-AP, pyroglutamate-aminopeptidase (EC 3.4.11.8).
* n.t., not tested.

The values in parentheses are the theoretical numbers of residues based on the composition of the proposed sequence. The data are averages of 2-3 determinations.

Oxytocin—HPLC fractionation of oxytocin digests showed a more complex profile. A 3-h incubation of the peptide with brain membranes resulted in the accumulation of at least nine components numbered I to IX (OXT-I to OXT-IX) in Fig. 3A. Fraction OXT-I co-eluted with intact oxytocin. ([14C]-Gly[NH2]2)-oxytocin digests showed that all fractions contained [14C]glycinamide radioactivity (Fig. 3B). HPLC of ([14C]-Tyr2)-OXT showed that the main [14C]tyrosine radioactivity was present in fractions OXT-I and OXT-III and at the elution position of free tyrosine (Fig. 3C).

Fractions OXT-IX to OXT-IX were subjected to chemical analysis. The amino acid composition, dansyl-residues, [14C] radioactivity, and co-migration with oxytocin demonstrated that fraction OXT-I to intact oxytocin (OXT-1-9) (Table III). Fraction OXT-II appeared heterogenous and no conclusive characterization was achieved for the peptides in this minor fraction. Fraction OXT-III differed from fraction OXT-I only in dansylation of the tyrosine residues. Fraction OXT-III yielded didansyl-tyrosine upon microdansylation, instead of the o-dansyl-tyrosine derivative as in OXT-1-9. It was concluded that fraction OXT-III contained the open nonapeptide Cys1 Tyr2-Ile-Gln-Asn-Cys6-Pro-Leu-Gly2NH2. The peptide in fraction OXT-IV was characterized as Ile2-Gln-Asn-Cys6-Pro-Leu-Gly2NH2. Synthetic [Cys6]-OXT-(2-9) co-migrated with fraction OXT-IV upon HPLC. The amino acid compositions of fractions OXT-V and OXT-VI, which were purified by rechromatography by HPLC under isocratic conditions (8% methanol in 10 mM ammonium acetate, pH 4.15), appeared to be similar and to fit the [Cys6]-OXT-(4-9) sequence. Upon dansylation, both fractions showed the monodansyl-cystine derivative. However, a dansyl-glutamic acid residue was found only in fraction OXT-VI. After treatment with pyroglutamate-aminopeptidase (EC 3.4.11.8) and dansylation, fraction OXT-V showed a dansyl-aspartic acid derivative (Table III). It was concluded that the peptide in OXT-VI was Glu1-Asn-Cys6-Pro-Leu-Gly2NH2 and the one in fraction OXT-V is typroglutamyl-Cys1 Tyr2-Ile-Gln-Asn-Cys6-Pro-Leu-Gly2NH2. Moreover, fraction OXT-V co-migrated with synthetic [Glu1, Cys6]-OXT-(4-9) on HPLC, while the elution of fraction OXT-VI at earlier retention times was compatible with the more hydrophobic nature of [Cys6]-OXT-(4-9), having a free NH2 terminus. After rechromatography of fraction OXT-VI, the material appeared heterogeneous and not related to the structure of oxytocin. Rechromatography of fraction OXT-VII by HPLC at 8% methanol yielded a small component with amino acid composition, NH2-terminal groups, and radioactivity content of Asn1-Cys6-Pro-Leu-Gly2NH2. This fraction co-migrated with synthetic [Cys6]-OXT-(6-9) on HPLC. Fraction OXT-IX was separated into three subfractions during rechromatography on HPLC at 2% methanol in 10 mM ammonium acetate, pH 4.15. The subfractions were rich in glycine residues, contained all amino acids common to protein hydrolyates, and were devoid of [14C]tyrosine and [14C]glycinamide radioactivity, which indicated that fraction OXT-IX did not contain a major oxytocin fragment. The data are summarized in Table III.

Time Course of Proteolysis of Arginine-Vasopressin and Oxytocin

In order to investigate the course of proteolytic events, arginine-vasopressin and oxytocin with their [14C]tyrosine- or [14C]glycinamide-labeled congeners were incubated with the synaptic membrane preparation and aliquots were taken at various time intervals. The samples were subjected to HPLC analysis and the metabolites were quantitated by their radioactivity content.
half-life of 40 min as calculated by linear regression analysis of the semilogarithmically plotted data. During proteolysis, free tyrosine was rapidly generated and estimated to be approximately 140 pmol/min/ml of incubation medium during the first 2 h of incubation (Fig. 4A). The release of glycaminamide was much slower and estimated to be approximately 13 nmol/min/ml of incubation medium. The accumulation of [Cyt'']-AVP-(2-9), which was measured by both its [14C]tyrosine and [14C]glycinamide radioactivity, was maximal at t = 40 min. [Cyt']-AVP-(3-9) appeared later and was maximal at t = 80 min (Fig. 4B). [Cyt', Cyt'']-AVP-(4-9) was the third product to be formed and its accumulation was maximal at t = 180 min.

14C-labeled AVP-(1-9), [Cyt']-AVP-(2-9), and [14C]tyrosine constituted 95% of the total 14C radioactivity during the time course. The combined recovery of 14C-containing fractions which eluted at 4 min/ml of incubation medium. The combined recovery of [14C]GlyNH2-OXT-(4-9), and [14C]glycinamide radioactivity, was maximal at t = 40 min to 46% at t = 240 min due to the formation of two 14C-containing fractions which eluted at 4 and 6 min in HPLC (Fig. 2B). These fractions likely contain the aminopeptidase split products [Cyt']- AVP-(4-9) and [Cyt']-AVP-(5-9). Including these fractions, the recovery was 86% at t = 240 min.

Under identical conditions, oxytocin had a calculated half-life of 200 min while tyrosine was generated with an initial velocity of approximately 50 pmol/min/ml of incubation medium (Fig. 5A). Glycinamide was generated at 10 pmol/min/ml of incubation medium in the initial phase. [Cyt']-OXT-(2-9) was formed as the initial product and was followed by [Cyt']-OXT-(3-9) (V), [<Glu', Cyt'']-OXT-(4-9) ( ), [Cyt']-OXT-(4-9) (V), [Cyt']-OXT-(5-9) (+), free tyrosine (), and free glycaminamide () were quantitated by the 14C radioactivity and expressed as nanomoles/ml of incubation medium. The data have not been corrected for the recovery. The recovery of oxytocin was 95.8% ± 1.2% at t = 0 min. The presented symbols are identical with those in Fig. 3 defining the components as obtained by HPLC fractionation.

86% at t = 240 min.

DISCUSSION

The present study has provided evidence that the predominant mechanism of proteolysis of arginine-vasopressin and oxytocin by brain synaptic membranes involves sequential
cleavage of peptide bonds from the NH₂ terminus (Fig. 6). This type of cleavage is characteristic for aminopeptidase activity. The evidence comes from three lines of experimental data. Firstly, the nature of the characterized peptide fragments of both arginine-vasopressin and oxytocin demonstrates that cleavage has occurred in the NH₂-terminal portion of the peptides and suggests a precursor-product inter-relationship between the metabolites. The peptides [Cyt⁴⁴]-AVP-(2-9) and [Cyt⁴⁴]-OXT-(2-9) are generated by a single cleavage of the cysteinyl¹-tyrosyl¹ bond of arginine-vasopressin and oxytocin, respectively. The presence of [Cyt⁴⁴]-AVP-(3-9) and [Cyt⁴⁴]-OXT-(3-9) indicates further cleavage of the neighboring tyrosyl²-phenylalanyl⁴ and tyrosyl²-isoleucyl⁴ bonds, respectively. The peptides having the NH₂-terminal pyroglutamyl acid or glutamine at position 4, such as in the characterized fragments [Glu¹-Cyt²]-AVP-(4-9), [Glu¹-Cyt²]-OXT-(4-9), and [Cyt²]-OXT-(4-9), or having NH₂-terminal asparagine as in [Cyt²]-OXT-(5-9) suggests that the proteolysis proceeds. Secondly, the time course studies, which show the consecutive cleavage of the cysteinyl¹-tyrosyl¹ bond of lysine-vasopressin by minced cortical tissue. Recently, we demonstrated that the release of tyrosine from oxytocin by brain synaptic membrane preparations was rapid as compared to the release of glycineamide (27, 43) and we tentatively characterized two oxytocin fragments which were indicative of aminopeptidase action (28). The characterization of a complete set of aminopeptidase split products and their precursor-product inter-relationship presented in this paper directly demonstrates the aminopeptidase mechanism. A noted feature of all split products is the conserved disulfide bridge between cysteine residues 1 and 6. This shows that the aminopeptidase activity cleaves the intact nonapeptides without prior reduction of the disulfide bridge. In this respect, the brain synaptic membrane aminopeptidase activity resembles purified “plasma oxytocinase,” an aminopeptidase-like enzyme circulating in the plasma of pregnant women (44, 45). However, aminopeptidases purified from brain tissue required the presence of sulfhydryl reducing agents or did not cleave oxytocin at all (46, 47).

From the present experiments, it appears that arginine-vasopressin and oxytocin are both converted similarly by the aminopeptidase activity. This is evident from the structure of the early fragments. Although the mechanism of conversion is similar, marked differences in the conversion rates were found. Oxytocin was more resistant to proteolytic cleavage than arginine-vasopressin; the half-life of intact oxytocin was about 5 times longer than that of arginine-vasopressin under identical experimental conditions. Recent studies on the conversion of oxytocin and vasopressin analogues showed that this difference in susceptibility to aminopeptidase cleavage is primarily determined by the amino acid residue in position 3 (isoleucine or phenylalanine) (43).

Most of the characterized peptides with sequences starting at residue 4 contained pyroglutamic acid in this position. The isolation of [Glu¹-Cyt²]-OXT-(4-9) separately from [Cyt²]-OXT-(4-9) indicated that formation of pyroglutamic acid had taken place before HPLC fractionation of the digests, i.e., during incubation or during sample preparation. Glutamine residues at the NH₂ terminus of peptides are known to cyclize readily. This process takes place spontaneously (48-50) but also enzymatic catalysis of the cyclization reaction has been recognized (51). Moreover, the presence of a pyroglutamic acid residue is a general feature of many biologically active peptides (52, 53). This residue protects peptides against attack of most aminopeptidases and it can contribute to or be prerequisite for the biological activity of peptides (46, 49, 54, 55). It is anticipated that the protective effect of pyroglutamic acid formation favors the accumulation of [Glu¹-Cyt²]-OXT-(4-9) and of [Glu¹-Cyt²]-OXT-(4-9), which exceeds that of [Cyt²]-OXT-(4-9) markedly. In addition, one may speculate that this pyroglutamic acid residue is of significance in that it attributes a biological activity to the formed fragment.

Arginine-vasopressin and oxytocin have been considered candidates to serve precursor functions for small neuropeptides with more specific functions in the brain (20, 23). In particular, this concept has been corroborated by studies on the effects of synthetic fragments on behavioral paradigms (20, 24, 56). Our experiments present the structure of the predominant metabolites formed by brain enzymes and delineate the mechanism of their formation. Preliminary observations indicate that these peptides have central activities in extremely low doses, thus indicating the physiological significance of the proteolytic process. The enzymes involved may have a role in the modulation of the central effects of arginine-vasopressin and oxytocin and their fragments.
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