Carboxypeptidase E (CPE): Immunocytochemical Localization in the Rat Central Nervous System and Pituitary Gland

David R. Lynch,^{1,a} Karen M. Braas,^{1,b} John C. Hutton,² and Solomon H. Snyder¹

¹Departments of Neuroscience, Pharmacology, and Molecular Sciences and Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, and ²Department of Clinical Biochemistry, University of Cambridge, Cambridge, England

Carboxypeptidase E (CPE, enkephalin convertase, carboxypeptidase H; EC 3.4.17.10) is a carboxypeptidase B-like enzyme proposed to be involved in the synthesis of a variety of peptide hormones and neurotransmitters. In the present study we have localized CPE in the rat central nervous system using specific polyclonal antisera to the purified enzyme. Immunoreactive CPE occurs in a variety of cell somas and neuronal terminals in the central nervous system. The enzyme is most concentrated in the hypothalamus, especially in the median eminence, supraoptic nucleus, paraventricular nucleus, and suprachiasmatic nucleus. CPE is also found in axon terminals in the posterior pituitary gland, melanotrope of the intermediate pituitary gland, and select cells of the anterior pituitary gland. Immunoreactivity to CPE in the hippocampus is found in the pyramidal cells and in the inner part of the molecular layer of the dentate gyrus. CPE is also found in the central nucleus of the amygdala and the bed nucleus of the stria terminalis. These results suggest that CPE distribution corresponds to that of many neuropeptides at the fine structural level, thus further implicating CPE in peptide processing.

Peptide hormones and neurotransmitters are commonly synthesized as precursors in which the bioactive peptide is flanked by dibasic amino acids (Docherty and Steiner, 1982). Thus, the action of a trypsin-like enzyme followed by a carboxypeptidase B-like enzyme is capable of producing the active peptide from the precursor molecule. Carboxypeptidase E (CPE, enkephalin convertase, carboxypeptidase H; EC 3.4.17.10) is a carboxypeptidase B-like enzyme whose properties suggest that it is involved in the processing of enkephalins and other neuropeptides *in vivo*. CPE has been purified from adrenal gland (Fricker and Snyder, 1982; Fricker et al., 1982), brain, pituitary gland (Fricker

and Snyder, 1983), and insulinoma cells (Docherty and Hutton, 1983; Davidson and Hutton, 1987), and is similar in all of these tissues. CPE is found in both membrane-associated and soluble forms, which differ in molecular weight by approximately 2 kDa, but have similar catalytic properties (Supattapone et al., 1984). CPE cleaves arginine or lysine residues from the carboxyl terminus of peptides, but does not remove other amino acids, nor does it have endopeptidase activity (Fricker and Snyder, 1982, 1983). However, CPE cleaves the basic carboxyl terminal amino acid regardless of the penultimate amino acid, and thus, CPE is not biochemically specific for any particular precursor. Consequently, the relative specificity of CPE *in vivo* should reflect the colocalization of the enzyme with specific peptide precursor molecules.

CPE is potently inhibited by guanidinoethylmercaptosuccinic acid (GEMSA; Fricker et al., 1983). Since the K_i of GEMSA for CPE is less than 10 nM, while its K_i values for other carboxypeptidase B-like enzymes are greater than 1 μ M, GEMSA interacts selectively with CPE at low nanomolar concentrations. ³H-GEMSA binds selectively to CPE (Strittmatter et al., 1984a), permitting autoradiographic localization (Lynch et al., 1984, 1986, 1987, 1988; Strittmatter et al., 1985, 1986). These techniques have localized CPE in endocrine cells of multiple organs, including the pituitary gland, islets of Langerhans in the pancreas, the cardiac atrium, and the adrenal medulla. In the central nervous system, CPE is found in a variety of putative neuropeptide-containing cells, as well as in cells not presently known to synthesize specific neuropeptides. Preliminary immunocytochemical studies showed high levels of CPE in the rat pituitary and hypothalamus and bovine adrenal medulla (Hook et al., 1985).

Polyclonal antisera have recently been produced against CPE from insulinoma cells (P. A. Guest, M. Ravazzola, L. Orci, and J. C. Hutton, unpublished observations). In the present study we have used these antisera to examine immunoreactive CPE in the central nervous system with an emphasis on the fine structural localization of the enzyme.

Materials and Methods

Antibody preparation. Antiserum was prepared using purified rat insulinoma cell secretory granule CPE (carboxypeptidase H) as described previously (Guest et al., unpublished observations). This antiserum recognizes 2 bands with apparent molecular weights of 53 kDa and 55 kDa in rat insulinoma, neurointermediate and anterior pituitary, and adrenal, consistent with membrane-associated and soluble CPE found in these tissues (Guest et al., unpublished observations). The immunoreactive material in these tissues is also found primarily in the secretory granules by Western blot (Guest et al., unpublished observations) and

Received May 2, 1989; revised Nov. 6, 1989; accepted Nov. 21, 1989.

This work was supported by U.S. Public Health Service grants DA-00266, NS-16375, and Research Scientist Award DA-00074 to S.H.S., training grant GM-07309 to D.R.L., and grants of the British Diabetic Association and the Wellcome Trust to J.C.H. We wish to thank Dr. Victor May for helpful input throughout this work and manuscript preparation, and Naomi Taylor for excellent technical assistance.

Correspondence should be addressed to Dr. Solomon H. Snyder, Departments of Neuroscience, Pharmacology and Molecular Sciences and Psychiatry, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.

^a Present address: Department of Neurology, Hospital of the University of Pennsylvania, 3400 Spruce, Philadelphia, PA 19104.

^b Present address: Department of Anatomy and Neurobiology, University of Vermont, Given Health Science Center, Burlington, VT 05405.

Copyright © 1990 Society for Neuroscience 0270-6474/90/051592-08\$02.00/0

Figure 1. Specificity of CPE immunocytochemistry. Paraffin-embedded tissue sections (10 μ m) were immunocytochemically stained with anti-CPE as described in the Materials and Methods. *A*, A section of the rat hippocampal formation stained with 1:2000 anti-CPE; *B*, an adjacent section stained with 1:2000 anti-CPE preabsorbed with 50 ng/ml purified rat pituitary CPE; *C*, an adjacent section stained with 1:2000 preimmune serum to replace the primary antiserum; *D*, a section stained with 1:2000 anti-CPE from which the biotinylated goat anti-rabbit IgG was omitted. Staining is intense around the inner portion of the molecular layer of the dentate gyrus (arrowheads) in *A*, but is abolished in *B*, *C*, and *D*. A lower level of staining is observed in the rostral hippocampus (*h*) as seen in *A*, but not observed in the other sections shown. *th*, Thalamus; *v*, third ventricle.

the antiserum selectively precipitates radiolabeled material of the proper molecular weight in islet cell cultures (Guest et al., 1989). The antiserum does not recognize any bands in Western blots of brain tissue, presumably because the brain contains only about 2–5% as much carboxypeptidase E as the pituitary (Fricker et al., 1982; Fricker and Snyder, 1983; Strittmatter et al., 1984b).

Tissue preparation for immunocytochemistry. Adult male Sprague-Dawley rats (225–250 gm) under sodium pentobarbital anaesthesia were perfused through the left cardiac ventricle with 150 ml of 100 mM sodium phosphate buffer, pH 7.4, containing 150 mM sodium chloride, followed by 250 ml of fixative in 150 mM sodium phosphate buffer, pH 7.4. For most experiments, tissues were fixed with 4.0% paraformaldehyde containing 0.1% glutaraldehyde, although for some experiments, 1.0% or 2.5% glutaraldehyde fixation was utilized. Following perfusion, the brain and pituitary gland were removed and immersed in the same fixative for 12 hr. The tissues were washed using 150 mM sodium phosphate buffer, pH 7.4, containing 0.32 M sucrose for 12 hr, and embedded in either paraffin or Araldite as described previously (Braas et al., 1987).

Paraffin-embedded tissues were sectioned (10 μ m) and mounted on chrome alum/gelatin subbed slides. For light microscopic studies, Araldite-embedded tissues were sectioned (0.2 μ m) using a Sorvall MT-2 ultramicrotome and mounted on chrome alum/gelatin coated slides. For electron microscopic studies, light gold interference (90 nm) tissue sections were cut using a diamond knife and mounted on nickel 200-mesh grids.

Light microscopic immunocytochemical staining. Paraffin-embedded tissue sections were stained using a modification of the avidin-biotin-peroxidase complex (ABC) technique (Hsu et al., 1981; Braas et al., 1986, 1987). Tissue sections were deparaffinized by incubation in xylene for 10 min, incubated in 0.3% hydrogen peroxide in absolute ethanol for 20 min to destroy endogenous peroxidase activity, and then rehydrated through a graded series of ethanol. The sections were incubated in 50 mM sodium phosphate buffer, pH 7.4, containing 4.0 mg/ml human serum albumin, 0.1% Triton X-100, and 150 mM sodium chlo-

ride (buffer A) for 15 min. The sections were rinsed briefly with buffer A and incubated with 1:1000 to 1:5000 anti-CPE in buffer A for 72 hr at 4°C. Following incubation, the sections were rinsed with buffer A and incubated for 90 min with 1:400 biotinylated goat anti-rabbit IgG. The sections were washed with buffer A for 15 min and then incubated with 1:200 avidin-biotin-peroxidase complex for 90 min. The sections were rinsed with buffer A without Triton X-100 for 15 min and the peroxidase reaction was performed using diaminobenzidine and hydrogen peroxide as chromophores. The sections were washed for 16 hr and exposed to osmium tetroxide vapors for 10 min. Finally, the tissue sections were dehydrated and coverslipped using Permount.

Araldite-embedded tissue sections were prepared for immunocytochemistry by etching for 10 min in 1:1 saturated alcoholic sodium hydroxide in absolute ethanol. Endogenous peroxidase activity was inactivated by incubation of the sections with 1.0% hydrogen peroxide in absolute ethanol for 10 min, and the tissue was rehydrated through a graded series of ethanol. The ABC immunocytochemical technique was then performed as described above for paraffin-embedded tissues.

Immunocytochemical staining control studies included normal rabbit serum or preimmune serum in place of the primary antibody, omission of the biotinylated goat anti-rabbit IgG, or omission of the ABC complex. Rat pituitary CPE and rat insulinoma cell CPE were purified as previously described (Davidson and Hutton, 1987; Lynch et al., 1987). Antiserum was preabsorbed with 10–200 ng/ml of purified CPE prior to the immunocytochemical stain. The present studies present only immunoreactivity that is blocked by purified rat islet cell and rat pituitary CPE.

Electron microscopic immunocytochemical staining. Ultrathin tissue sections were stained using a modification of the ABC technique (Larsen, 1983; May et al., 1987). Briefly, tissue sections were etched with 10.0% hydrogen peroxide for 10 min followed by incubation with buffer A for 3 min. The tissue was incubated with 1:2000 primary antibody for 24 hr at 4°C. Following a wash with buffer A, the tissue was incubated with 1:100 biotinylated goat anti-rabbit IgG in buffer A for 3 min. The

Figure 2. Immunoreactive CPE in the median eminence of the rat brain. Paraffin-embedded tissue sections (10 μ m) of rat brain at the level of the median eminence and pituitary stalk were immunocytochemically stained with 1:5000 anti-CPE as described in Materials and Methods. The median eminence (*me*) is intensely stained for CPE (*A*). Staining is present mainly in the outer zone of the median eminence and is closely associated with the vessels at the surface of the brain (*B*) (*v*, third ventricle). The pituitary stalk (*ps*) also contains high levels of immunoreactive CPE (*C*). A few immunoreactive fibers are detectable in the medial hypothalamus at this brain level (*arrowheads*). Staining is absent from the median eminence when stained with preimmune serum (*D*). The fine structure of the CPE-like immunoreactivity in the median eminence is shown in *E* (araldite-embedded tissue, 2 μ m). The staining in the outer zone of the median eminence appears to be arranged longitudinally along the vessels passing through the region (*arrowheads*).

Figure 3. CPE-like immunoreactivity in the hypothalamus. CPE-like immunoreactivity is found in a variety of locations in the hypothalamus. The highest level of immunoreactivity is in the supraoptic nucleus (*so*), where staining is perinuclear in the cell bodies (*A*, *B*). No staining is observed in the blood vessels (*bv*, arrows *C*, *D*) or the optic tract (*op*). A similar pattern for CPE is observed autoradiographically in the hypothalamus using ^3H -GEMSA. A high density of autoradiographic grains is observed in the darkfield micrograph (*D*) over the supraoptic nucleus seen in the bright-field micrograph (*C*). Staining is also found in the suprachiasmatic nucleus (*sc*) of the hypothalamus (*E*), but not in the optic chiasm (*ox*). Immunoreactive CPE is also present in fibers in the lateral hypothalamus at the level of the median eminence (*F*), where they overlie the optic tract and appear to course medially (*m*). *n*, Nucleus; *m*, midline of the brain.

Figure 4. CPE localized in the magnocellular nuclei of the hypothalamus. Araldite-embedded tissue sections ($0.25\ \mu\text{m}$) through the level of the hypothalamus were immunocytochemically stained with anti-CPE. In the supraoptic nucleus (so; *A–D*), perinuclear staining is observed in the cell bodies; a weak nonspecific staining is found in the optic tract (op). Numerous fibers are also stained within the supraoptic nucleus (arrowheads). *C* and *D*, A cell in which a fiber appears to emerge from the stained portion of a cell body (arrowhead). Similar cells and fibers are also found in the magnocellular portion of the paraventricular nucleus (*E*), where an immunoreactive fiber clearly joins an immunoreactive neuron (arrowhead).

tissue was washed with buffer A and incubated in 1:100 ABC in buffer A for 3 min, and washed with 50 mM Tris-HCl, pH 7.6. The peroxidase reaction was performed using diaminobenzidine and hydrogen peroxide as substrates for 3 min. The tissue was washed, incubated with 4.0% osmium tetroxide for 10 min, and washed thoroughly with water.

Tissue preparation for autoradiography. Adult male Sprague-Dawley rats under sodium pentobarbital anesthesia were perfused through the left cardiac ventricle with Hanks' balanced salt solution followed by 0.32 M buffered sucrose. The brains were embedded in brain paste and frozen as previously described (Braas et al., 1987). Cryostat tissue sections ($8\ \mu\text{m}$) were mounted onto chrome alum/gelatin-coated slides and stored at -20°C .

Enzyme labeling and autoradiography. Binding of ^3H -GEMSA (30 Ci/mmol) (New England Nuclear-DuPont, Boston, MA) to tissue sections was performed as described previously (Lynch et al., 1984, 1986, 1987, 1988). The labeled sections were apposed to Kodak NTB-3 emulsion-coated coverslips (Braas et al., 1987) for 14 d.

Results

Characterization of the CPE antiserum

The antiserum used in the present study has previously been shown to stain bands of 55 and 57 kDa in Western blot analysis of insulinoma tissue, which correspond to soluble and membrane-associated CPE (Guest et al., unpublished observations). This antiserum has been used to immunocytochemically stain a variety of regions in the rat brain. The optimal antiserum dilution for immunocytochemical staining of paraffin-embedded tissue is 1:2000, although staining is still readily detectable with dilutions up to 1:5000. Staining in all regions is reduced by approximately 50% and more than 90% by preabsorption of 1:2000 anti-CPE, respectively, with 10 ng/ml and 50 ng/ml of

purified rat pituitary CPE (Fig. 1*B*). Staining is also inhibited by preabsorption with 200 ng/ml of purified insulinoma cell CPE. Omission of the biotinylated anti-rabbit IgG (Fig. 1*C*) or the ABC abolishes all staining. A low level of nonspecific staining is observed with 1:2000 dilutions of primary antiserum or normal rabbit serum (Fig. 1*D*).

Distribution of immunoreactive CPE in the rat brain

Immunoreactive CPE is detectable in a variety of central nervous system regions. Highest levels of staining are found in association with the peptidergic systems of the hypothalamus. The outer zone of the median eminence contains the densest collection of CPE-like immunoreactivity, found mainly in terminals and fibers in the outer zone (Fig. 2, *A*, *B*), which corresponds closely with the distribution of CPE observed by autoradiography using ^3H -GEMSA.

CPE-like immunoreactivity is also found in the pituitary stalk (Fig. 2*C*). The immunoreactivity appears to run longitudinally along the vessels in some regions of the pituitary stalk, but the vessels themselves are not stained (Fig. 2*E*).

Substantial CPE-like immunoreactivity is observed in the supraoptic and paraventricular nuclei of the hypothalamus (Figs. 3 and 4). Here, CPE is found mainly in the magnocellular cell somas of these nuclei, but also in large axon-like fibers, which in some cases can be seen to arise from the magnocellular cell bodies. Since CPE is also found in the pituitary stalk and in fibers in the posterior pituitary (Fig. 7*B*), immunoreactive CPE

Figure 5. Localization of immunoreactive CPE in the hippocampal formation. Paraffin tissue sections (10 μ m) at the level of the hippocampus were immunocytochemically stained with 1:1000 anti-CPE as described in Materials and Methods. Immunoreactivity is found in the hippocampal pyramidal cells (*py*) of CA3, and in fibers in the inner portion of the molecular layer (*mol*) of the dentate gyrus (*A, B, E–G*). Immunoreactivity is largely absent in the adjacent thalamus (*th*). This matches the pattern of CPE observed with 3 H-GEMSA autoradiography. *D* shows the localization of 3 H-GEMSA binding over the tissue section shown in *C*. Autoradiography, CPE levels are highest (*white areas*) over the pyramidal cells and over the inner portion of the dentate gyrus molecular layer. At a higher magnification, the CPE-like immunoreactivity is perinuclear in the pyramidal cells (*E*). In the dentate gyrus, the immunoreactivity is found in fine processes in the inner portion of the molecular layer (*F, G*), while the granule cells (*gr*) have little to no immunoreactivity. *rad*, Stratum radiatum of the hippocampus; *or*, stratum oriens.

can be traced within all components of the hypothalamic-neurohypophyseal system.

CPE-like immunoreactivity also occurs in other select regions of the hypothalamus. The suprachiasmatic nucleus contains both immunoreactive fibers and cell somas, with the cell bodies concentrated medially in the nucleus (Fig. 3*E*). Immunoreactive fibers en passant are detected in the lateral hypothalamus at the level of the median eminence, where they appear to course toward the median eminence (Fig. 3*F*).

High levels of CPE are detectable in the hippocampal for-

mation (Fig. 5). The pyramidal cells of the hippocampus stain prominently for CPE, especially in the CA3 region (Fig. 5, *B, F*). Fibers in the inner portion of the molecular layer of the dentate gyrus are stained, while the stratum oriens and radiatum have no detectable immunoreactive fibers. Staining is weak over the granule cell layer, and in the outer portion of the molecular layer (Fig. 5, *A, F*). The distribution of the CPE-like immunoreactivity in the hippocampal formation is identical to that of CPE labeled by 3 H-GEMSA (Fig. 5, *C, D*).

Immunoreactive CPE can be detected in a few other selected

Figure 6. Immunocytochemical localization of CPE in the bed nucleus of the stria terminalis and the amygdala. Immunoreactive CPE is localized to fine fibers in the bed nucleus of the stria terminalis (*bst*) (*A, B*). Little immunoreactivity is found in the fornix (*f*) or the internal capsule (*ic*). In the central nucleus of the amygdala, immunoreactivity is found in fibers (*arrowheads*) and occasional cell bodies (*C*).

Figure 7. Localization of CPE in the rat pituitary gland. Araldite-embedded tissue sections ($0.25\ \mu\text{m}$) from all 3 pituitary lobes were immunocytochemically stained with 1:2000 anti-CPE as described in Materials and Methods for light microscopy or with 1:2000 anti-CPE for electron microscopy. Immunoreactivity is observed in all melanotrope of the intermediate lobe (*ip*) (*A* and *C*), and in fibers and Herring bodies (arrowheads) in the posterior lobe (*pp*) (*A* and *B*). Specific cells (arrowheads) of the anterior pituitary stain, while the endothelial cells of the blood vessels (*bv*) are unstained (*D*). At the electron microscopic level, CPE-like immunoreactivity is localized to the secretory granules (small arrowheads) of cells in the anterior pituitary (*D*, inset) (*n*, nucleus), while unstained granules appear clear. The staining varies in the different granules from light gray to dark.

regions of the rat brain. These include the bed nucleus of the stria terminalis and the central nucleus of the amygdala (Fig. 6). The medulla, cerebellum, pons, midbrain, thalamus, and remainder of the forebrain do not contain reproducibly detectable CPE-like immunoreactivity.

Localization of CPE in the rat pituitary gland

CPE-like immunoreactivity is observed in all 3 lobes of the rat pituitary gland (Fig. 7). Highest levels are found in the intermediate pituitary gland, where CPE-like immunoreactivity displays a punctate cytoplasmic localization consistent with localization to secretory granules in melanotrope (Fig. 7, *A*, *C*). The high level of CPE-like immunoreactivity in the intermediate pituitary gland agrees with its localization by ^3H -GEMSA autoradiography. In the posterior pituitary gland, CPE occurs in a punctate pattern suggesting localization to nerve fibers, as well as in larger collections resembling collections of transmitter called Herring bodies (Fig. 7, *A*, *B*). CPE is also found in select cells in the anterior pituitary gland (Fig. 7*D*). At the electron microscopic level, these cells morphologically resemble the classical follicle stimulating hormone containing gonadotrope type I cell (Fig. 7*D*, inset). Within these cells, immunoreactive CPE is localized to both large- and small-diameter secretory granules. Further studies using antisera against pituitary hormones would be necessary to directly determine the cell type(s) expressing CPE.

Discussion

The present study immunocytochemically localizes CPE throughout the central nervous system in neuronal cell bodies,

fibers, and terminals. In a preliminary study, staining for CPE was observed in cell bodies of the supraoptic and paraventricular nuclei of the hypothalamus and the anterior and intermediate lobes of the pituitary gland (Hook et al., 1985). Unlike the present results, the previous study did not report staining for CPE in the neural lobe of the pituitary gland. The CPE distribution generally resembles autoradiographic localizations using ^3H -GEMSA (Lynch et al., 1984, 1986), confirming the selectivity of our immunocytochemical localizations. The antiserum used in this study, however, demonstrates immunoreactive CPE in fewer areas than does the autoradiographic procedure (Table 1). With the exception of the lateral septum, all areas with levels of ^3H -GEMSA binding greater than 1.6 pmol/mg protein exhibit immunoreactive CPE (Lynch et al., 1984, 1986). The dentate gyrus and the paraventricular nucleus of the hypothalamus have slightly lower levels of ^3H -GEMSA binding but detectable immunoreactivity, apparently because the immunoreactivity is found in a select anatomical distribution within these nuclei. Discrepancies between the 2 techniques of enzyme localization probably reflect the greater sensitivity of ^3H -GEMSA autoradiography. Angiotensin converting enzyme is more widely distributed when examined by ^3H -captopril autoradiography than by immunocytochemistry, and similar results are observed for enkephalinase and kininase (Defendini et al., 1983; Strittmatter et al., 1984b, 1987; Correa, 1986; Matsas et al., 1986; Waksman et al., 1986; Chai et al., 1987). Apart from the differences in sensitivity between the techniques, GEMSA binding and immunocytochemistry may detect slightly different sites. Since the cDNA for CPE predicts a protein significantly larger than mature CPE (Fricker et al., 1986), it is possible that CPE immuno-

Table 1. Regional distribution of CPE-like immunoreactivity and ³H-GEMSA binding sites

Brain region	³ H-GEMSA bound (pmol/mg prot.)	Immunoreactivity
Lateral septum	2.3	—
Bed nucleus of the stria terminalis	2.0	+
Hippocampal pyramidal cells (CA3)	1.8	+
Amygdala		
Central	1.9	+
Anterior	1.0	—
Lateral	1.0	—
Medial	1.0	—
Median eminence	3.1	+
Hypothalamus		
Supraoptic	2.0	+
Preoptic	1.6	—
Paraventricular	1.4	+
Anterior	1.0	—
Medial basal	0.9	+

CPE immunocytochemistry was compared with the distribution of the enzyme as previously localized by ³H-GEMSA autoradiography (Lynch et al., 1984). Sections through all regions of the brain stem, thalamus, hypothalamus, and forebrain were examined for CPE-like immunoreactivity. Shown are only the areas that have CPE-like immunoreactivity and substantial levels of ³H-GEMSA binding. CPE immunoreactivity was not detected in the medulla, pons, midbrain, thalamus, and forebrain outside the hippocampus.

reactivity and GEMSA binding may not always be associated. Conceivably, the CPE precursor could be processed so as to separate the immunoreactive and GEMSA binding sites.

CPE is generally found in central nervous system cells associated with putative peptide neurotransmitter synthesis (Lynch et al., 1984, 1986). The cell somas of the supraoptic and paraventricular nuclei have immunoreactive CPE, as do their terminals in the posterior pituitary gland. CPE is also found in their fibers in the pituitary stalk. The cells in these nuclei synthesize the neuroendocrine peptides oxytocin and vasopressin, as well as proenkephalin B-derived peptides (Watson et al., 1982; Weber and Barchas, 1983). Very high levels of immunoreactive CPE are also found in terminals in the median eminence, where peptidergic hypothalamic releasing factors are secreted into the pituitary-hypophyseal portal circulation.

Immunoreactive CPE is demonstrable in a limited number of other locations within the central nervous system. The bed nucleus of the stria terminalis and the central nucleus of the amygdala contain substantial numbers of CPE immunoreactive fibers as well as ³H-GEMSA binding sites (Lynch et al., 1984, 1986). These nuclei contain high levels of several neuropeptides and the stria terminalis pathway contains neurotensin and enkephalin (Uhl et al., 1977, 1978).

In the hippocampus, however, CPE distribution differs from that of enkephalins and other known neuropeptides. Immunoreactive CPE as well as ³H-GEMSA binding is most prominent in the inner portion of the molecular layer of the dentate gyrus and in the pyramidal cells of CA3 (Lynch et al., 1984, 1986). However, most neuropeptides within the hippocampus are contained in the perforant pathway, which terminates in the outer portion of the molecular layer of the dentate gyrus and/or in the mossy fibers, which arise in the granule cells of the dentate gyrus and terminate in the stratum lucidum of CA3

(Gall et al., 1981; McGinty et al., 1983; Walaas, 1983; Chavkin et al., 1985). The different localization of CPE suggests that in the hippocampus, CPE may process as yet unknown substrates. The relative absence of CPE in the mossy fiber pathway implies either that processing in this pathway is relatively slow, or that another carboxypeptidase processes precursors such as proenkephalin B.

CPE is presently the only known carboxypeptidase B-like enzyme with selective localization in the central nervous system and endocrine tissues. With the exception of the hippocampal pyramidal cells, the regions expressing the enzyme also contain high levels of enkephalin or other neuropeptides. Still, in regions such as the striatum, where CPE levels are low by all available methods of detection although neuropeptide levels are high, other carboxypeptidase B-like enzymes may be active in processing.

References

- Braas KM, Newby AC, Wilson VS, Snyder SH (1986) Adenosine-containing neurons in the brain localized by immunocytochemistry. *J Neurosci* 6:1952–1961.
- Braas KM, Zarbin MA, Snyder SH (1987) Endogenous adenosine and adenosine receptors localized to ganglion cells of the retina. *Proc Natl Acad Sci USA* 84:3906–3910.
- Chai SY, Mendelsohn FA, Paxinos G (1987) Angiotensin converting enzyme in rat brain visualized by quantitative *in vitro* autoradiography. *Neuroscience* 20:615–627.
- Chavkin C, Shoemaker WJ, McGinty J, Bayon A, Bloom F (1985) Characterization of the prodynorphin and proenkephalin neuropeptide systems in rat hippocampus. *J Neurosci* 5:808–816.
- Correa FMA (1986) Quantitative distribution of angiotensin converting enzyme (kininase 2) in discrete areas of rat brain by autoradiography with computer microdensitometry. *Brain Res* 375:259–266.
- Davidson HW, Hutton JC (1987) The insulin-secretory-granule carboxypeptidase H. *Biochem J* 245:575–582.
- Defendini R, Zimmerman EA, Weare JA, Alhenc-Glass F, Erdos E (1983) Angiotensin-converting enzyme in epithelial and neuroepithelial cells. *Neuroendocrinology* 37:32–40.
- Docherty K, Hutton JC (1983) Carboxypeptidase activity in the insulin secretory granule. *FEBS Lett* 162:137–141.
- Docherty K, Steiner DF (1982) Post-translational proteolysis in polypeptide hormone biosynthesis. *Annu Rev Physiol* 44:625–638.
- Fricker LD, Snyder SH (1982) Enkephalin convertase: purification and characterization of a specific enkephalin-synthesizing carboxypeptidase localized to adrenal chromaffin granules. *Proc Natl Acad Sci USA* 79:3886–3890.
- Fricker LD, Snyder SH (1983) Purification and characterization of enkephalin convertase: an enkephalin synthesizing carboxypeptidase. *J Biol Chem* 258:10950–10955.
- Fricker LD, Supattapone S, Snyder SH (1982) Enkephalin convertase: specific enkephalin synthesizing carboxypeptidase in adrenal granules, brain and pituitary gland. *Life Sci* 31:1841–1844.
- Fricker LD, Plummer TH, Snyder SH (1983) Enkephalin convertase: potent selective and irreversible inhibitors. *Biochem Biophys Res Commun* 111:994–1000.
- Fricker LD, Evans CJ, Esch FS, Herbert E (1986) Cloning and sequence analysis of cDNA for bovine carboxypeptidase E. *Nature* 323:461–464.
- Gall C, Brecha N, Chang KJ, Karten HJ (1981) Localization of enkephalin-like immunoreactivity to identified axonal and neuronal population of the rat hippocampus. *J Comp Neurol* 198:335–350.
- Guest CJ, Rhodes CJ, Hutton JC (1989) Regulation of the biosynthesis of insulin-secretory-granule proteins. *Biochem J* 257:431–437.
- Hook VYH, Hezey E, Fricker LD, Pruss RM, Siegel RE, Brownstein MJ (1985) Immunochemical characterization of carboxypeptidase B-like peptide-hormone-processing enzyme. *Proc Natl Acad Sci USA* 82:4745–4749.
- Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. *J Histochem Cytochem* 29:577–580.

- Larsson L-I (1983) Methods for immunocytochemistry of neurohormonal peptides. In: *Handbook of chemical neuroanatomy*. Vol 1: *Methods in chemical neuroanatomy* (Bjorklund A, Hokfelt T, eds), pp 147–209. New York: Elsevier.
- Lynch DR, Strittmatter SM, Snyder SH (1984) Enkephalin convertase localization by guanidinoethylmercaptosuccinic acid autoradiography: selective association with enkephalin containing neurons. *Proc Natl Acad Sci USA* 81:6543–6547.
- Lynch DR, Strittmatter SM, Venable JC, Snyder SH (1986) Enkephalin convertase: localization to specific neuronal pathways. *J Neurosci* 6:1662–1675.
- Lynch DR, Strittmatter SM, Venable JC, Snyder SH (1987) Enkephalin convertase in the gastrointestinal tract and associated organs characterized and localized with [³H]-guanidinoethylmercaptosuccinic acid. *Endocrinology* 121:116–126.
- Lynch DR, Venable JC, Snyder SH (1988) Enkephalin convertase in the heart: similar disposition to atrial natriuretic factor. *Endocrinology* 121:116–126.
- Matsas R, Kenny AJ, Turner AJ (1986) An immunohistochemical study of endopeptidase-24.11 (“enkephalinase”) in the pig nervous system. *Neuroscience* 18:991–1012.
- May V, Wilber JF, U’Prichard DC, Childs GV (1987) GnRH and TRH immunoreactivities in primary rat anterior pituitary cell culture. *Peptides* 8:543–558.
- McGinty JG, Henrikson SJ, Goldstein A, Terenius L, Bloom F (1983) Dynorphin is contained within hippocampal mossy fibers: immunohistochemical alterations after kainic acid administration and colchicine induced neurotoxicity. *Proc Natl Acad Sci USA* 80:589–593.
- Strittmatter SM, Snyder SH (1987) Angiotensin converting enzyme immunohistochemistry in brain and pituitary gland: correlation of isozyme type with cellular localization. *Neuroscience* 21:407–420.
- Strittmatter SM, Lynch DR, Snyder SH (1984a) [³H]-guanidinoethylmercaptosuccinic acid binding to tissue homogenates: selective labeling of enkephalin convertase. *J Biol Chem* 259:11812–11817.
- Strittmatter SM, Lo MMS, Javitch JA, Snyder SH (1984b) Autoradiographic visualization of angiotensin converting enzyme in rat brain with [³H]captopril: localization to a striatonigral pathway. *Proc Natl Acad Sci USA* 81:1599–1603.
- Strittmatter SM, Lynch DR, DeSouza EB, Snyder SH (1985) Enkephalin convertase demonstrated in the pituitary and adrenal gland by [³H]-guanidinoethylmercaptosuccinic acid autoradiography: dehydration increases neurohypophyseal levels. *Endocrinology* 111:1667–1674.
- Strittmatter SM, Lynch DR, Snyder SH (1986) Differential ontogeny of rat brain peptidases: prenatal expression of enkephalin convertase and postnatal development of angiotensin converting enzyme. *Dev Brain Res* 29:207–215.
- Supattapone S, Fricker LD, Snyder SH (1984) Purification and characterization of a membrane bound enkephalin-forming carboxypeptidase, “enkephalin convertase.” *J Neurochem* 42:1017–1023.
- Uhl GR, Kuhar MJ, Snyder SH (1977) Neurotensin: immunohistochemical localization in rat central nervous system. *Proc Natl Acad Sci USA* 74:4059–4063.
- Uhl GR, Kuhar MJ, Snyder SH (1978) Enkephalin containing pathway: amygdaloid efferents in the stria terminalis. *Brain Res* 149:223–228.
- Waksman G, Hamel E, Fournie-Zaluski MC, Roques BP (1986) Autoradiographic comparison of the distribution of the neutral endopeptidase “enkephalinase” and μ and δ opioid receptors in rat brain. *Proc Natl Acad Sci USA* 83:1523–1527.
- Walaas I (1983) The hippocampus. In: *Chemical neuroanatomy* (Emsen PC, ed), pp 337–358. New York: Raven.
- Watson SJ, Khachaturian H, Akil H, Cox PH, Goldstein A (1982) Comparison of the distribution of dynorphin systems and enkephalin systems in brain. *Science* 218:1134–1136.
- Weber E, Barchas JD (1983) Immunohistochemical distribution of dynorphin B in rat brain: relation to dynorphin A and alpha-neoendorphin systems. *Proc Natl Acad Sci USA* 80:1125–1129.