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Exocrine secretion granules contain peptide amidation activity

(post-translational processing/parotid gland)

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ABSTRACT **Exocrine secretion granules from the rat parotid gland contain a carboxyl-terminal peptide α-amidation enzyme resembling closely an enzyme from the pituitary (peptidyl-glycine α-amidating monooxygenase) that functions in post-translational processing of secretory polypeptides within neural and endocrine secretion granules. α-Amidation is a characteristic (often essential) chemical feature of a variety of biologically active regulatory peptides in animals. The parotid and pituitary activities exhibit very similar ascorbate and copper requirements, pH dependence, and kinetic properties. Further, like the pituitary enzyme(s), the parotid activity is found predominantly in secretion granule content and is discharged by exocytosis. These results establish the presence of a novel enzyme in exocrine secretion granules and suggest a potential role of the L-ascorbic acid contained in parotid granules. Two additional findings—the detection of similar levels of amidation activity in purified secretion granule fractions from other exocrine glands and the observation, in parotid granule fractions, of a B-type carboxypeptidase activity similar to that involved in post-translational processing in other systems—form a rational basis for considering whether exocrine secretion granules (like their neural and endocrine counterparts) serve as post-translational processing sites. The identity and functional role of the modified polypeptides remain to be determined.**

Many biologically active secretory polypeptides, including mammalian hormones and neural peptides (e.g., gastrin, cholecystokinin, and substance P) have amidated carboxyl termini (1). This modification appears to be unique to regulatory peptides, and it has been used as a chemical marker for the discovery and isolation of several novel species with still unknown physiologic function (2–4). It is not known whether α-amidated peptides are also produced by mammalian exocrine systems, but such molecules have been detected in the lumen of the gastrointestinal tract [e.g., gastrin (5, 6)], and α-amidated peptides are secreted by exocrine tissues of lower animals (7–10).

Polypeptide α-amidation occurs as the final step in a series of post-translational modifications at the carboxyl terminus (11, 12). Following endoproteolysis (distal to paired basic residues) and exoproteolysis (to remove these basic residues) within Golgi and maturing secretion granule compartments, peptidyl-glycine products undergo amide formation in secretion granules. The reaction is catalyzed by an ascorbic acid-requiring enzyme, peptidyl-glycine α-amidating monooxygenase (13), and results in oxidative splitting of the terminal glycyl residue, leaving its nitrogen in an amidated carboxyl terminus on the preceding residue (11, 12).§ This enzyme has been detected and characterized as a soluble (content) component of neural and endocrine secretion granules isolated from anterior, intermediate, and posterior pituitary (12, 14). Further, pituitary secretion granules contain ascorbate at a concentration (1–3 mM) close to that which maximally stimulates activity in an *in vitro* assay (15, 16). Peptide amidation is thought to occur similarly in other neural and endocrine cells; however, an additional activity with a less stringent substrate specificity has been detected in brain (17).

Secretion granules isolated from an exocrine gland, the rat parotid, contain ≈1 mM ascorbate (18), but no physiologic function of this molecule in exocrine granules has been suggested to date. We report here that parotid granules also contain a soluble peptide carboxyl-terminal α-amidating activity having enzymatic properties closely resembling those of the pituitary enzyme(s). The luminal discharge of this activity in response to acinar cell stimulation and its presence in granules isolated from exocrine pancreas, submandibular, and lacrimal glands confirm its exocrine secretory origin. Part of these studies have been presented in abstract form (19).

MATERIALS AND METHODS

Isolation of Secretion Granules. Parotid glands were from male Sprague–Dawley rats (≈150 g; starved overnight), and secretion granule fractions were prepared at 4°C from tissue homogenates (5% wt/vol; as described in ref. 20) by three methods: (*i*) differential velocity sedimentation using a protocol similar to that outlined in ref. 21 in which the lower portion of the final granule pellet was collected selectively (18, 22); (*ii*) Percoll (Pharmacia) density gradient centrifugation of a postnuclear supernatant yielding a highly purified granule fraction (18); and (*iii*) centrifugation of a postnuclear supernatant on linear density gradients containing 0.3–2 M sucrose in 5% (wt/vol) Ficoll 400 (Pharmacia), as described (20). Aliquots of the latter gradient fractions were mixed with 8000 cpm of [^{14}C]dextran (70 kDa; New England Nuclear), dialyzed (2-kDa cutoff) in 20 mM sodium phosphate (pH 7.1), and concentrated against polyethylene glycol prior to amidation assay. [^{14}C]Dextran was recovered quantitatively and provided a measure of concentration (3- to 8-fold) for each fraction assayed.

Subcellular fractions were lysed by three freeze–thaw cycles. Lysates containing <300 mM sucrose (and negligible Percoll or Ficoll) were separated into membrane and soluble subfractions (20) by centrifugation at 100,000 × g_{av} for 10 min.

Highly purified secretion granule fractions, prepared by sucrose/Ficoll density gradient centrifugation, were kindly

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Abbreviations: D-Tyr-Val-NH_2, D-tyrosylvalinamide; Et_2NCS_2H, diethyldithiocarbamic acid; sodium Mops, sodium 3-(*N*-morpholino)propanesulfonate; BzPhe, benzoylphenylalanine.

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§The term "peptide amidation" will be used throughout to denote peptide amide formation by such a cleavage reaction rather than by the addition of an amide moiety to an existing carboxyl-terminal residue.

provided by R. Cameron (R. S. Cameron, P. L. Cameron, and J.D.C., unpublished data).

Collection of Parotid Secretion. *In situ.* Parotid ducts of 200-g rats were cannulated following intraperitoneal administration of isoproterenol (18).

From acini incubated in vitro. Collagenase-dissociated parotid acini (23) were incubated in Eagle's minimal essential medium (Earle's salts) for 60 min at 37°C in the presence or absence of 20 μM isoproterenol. Samples were collected at specified times for assay of secretion (α-amylase and amidating enzyme) and cell integrity (lactate dehydrogenase).

Peptide α-Amidation Assay. D-Tyr-Val-Gly (Bachem Fine Chemicals, Torrance, CA) was purified by reverse-phase HPLC (24). D-Tyrosylvalinamide (D-Tyr-Val-NH_2) was a generous gift of R. Mains (Department of Neuroscience, The Johns Hopkins University School of Medicine). Both peptides were radioiodinated (Iodo-Beads; Pierce) to obtain primarily the monoiodinated products, which were purified on sulfopropyl-Sephadex (Pharmacia) minicolumns (24). Fractions containing pure ^{125}I-labeled D-Tyr-Val-Gly, identified by TLC on silica (Kieselgel) in 4:1:1 (vol/vol) 1-butanol/acetic acid/water and visualized by autoradiography, were pooled as substrate for amidation assays.

Enzyme activity was measured as the production of ^{125}I-labeled D-Tyr-Val-NH_2 isolated on sulfopropyl-Sephadex minicolumns (24) and counted at ≈35% efficiency. Background (no enzyme or boiled enzyme) measured <0.5% conversion. The identity of the parotid granule reaction product was confirmed by TLC after substituting 10 mM pyridine acetate (pH 5.0) for the usual (24) 10 mM sodium phosphate (pH 5.0), eluting the product with 1 M pyridine acetate (pH 5.0), and lyophilizing the product fraction repeatedly prior to TLC analysis. As in the usual washing/elution protocol, parotid reaction product and synthetic ^{125}I-labeled D-Tyr-Val-NH_2 were recovered equally (82–86%), and assay background remained <0.5%.

Unless noted otherwise, peptide amidation activity was measured at pH 7.0. Bovine liver catalase (Sigma) was added routinely (0.1–0.5 mg/ml) to stabilize ascorbate (12, 14). Assays were performed in duplicate (agreement within 7% of the mean) under conditions in which linearity with time and amount of granule protein was obtained. Steady-state reaction velocity, expressed as pmol of D-Tyr-Val-NH_2 produced per hr at 37°C, was calculated at one or two time points.

Carboxypeptidase Assay. We used benzoylphenylalanine (BzPhe; Sigma) and Ala-Arg (Research Plus, Bayonne, NJ) to synthesize BzPhe-Ala-Arg (25), substituting BzPhe for dansylphenylalanine and using preparative TLC on Kieselgel in 1:1 (vol/vol) chloroform/methanol for final purification. The product cochromatographed with [*benzoyl*-2,5-^{3}H(N)]-BzPhe-Ala-Arg obtained commercially (30 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq). Parotid granule lysates (5–20 μg of protein) were assayed by a modification of (26) exactly as described (27). The extent of reaction (% conversion) is reported as the percentage of total radioactivity extractable into the chloroform phase after incubation with enzyme (27); background in the absence of added enzyme measured <2% conversion.

Biochemical Assays. α-Amylase activity was measured by the method of Bernfeld (28); lactate dehydrogenase activity was determined as described by Schnaar *et al.* (29); and protein was assayed using fluorescamine (30) with bovine serum albumin as the standard.

RESULTS

Detection of Peptide α-Amidation Activity in Isolated Parotid Secretion Granules. Freeze–thaw lysates of parotid secretion granule fractions, when incubated with ^{125}I-labeled D-Tyr-Val-Gly, formed a reaction product that was eluted from sulfopropyl-Sephadex at high salt concentration and that cochromatographed as a single radiolabeled spot with authentic ^{125}I-labeled D-Tyr-Val-NH_2 by TLC on silica (Kieselgel) with either acidic [4:1:1 (vol/vol) 1-butanol/acetic acid/water; R_f = 0.57) or basic (20:8:1 2-methyl-2-propanol/water/ammonia; R_f = 0.69) developing solvents.

Parotid granule fractions, prepared by either differential velocity centrifugation or Percoll gradient centrifugation, contained comparable levels of peptide amidation activity (Table 1). A more comprehensive analysis of the subcellular distribution of the parotid amidation activity was conducted on fractions from sucrose/Ficoll density gradients. This analysis (Fig. 1) confirmed that the amidation activity copurifies with secretion granules, marked by α-amylase (20, 31), throughout the granule peak (arrow). The peptide amidation/α-amylase activity ratio measured in the granule peak was 2.6 fmol·hr^{-1}·(units of amylase)$^{-1}$, similar to ratios found for purified secretion granule fractions prepared by the other procedures (Table 1). Most (>85%) of the peptide amidation activity detected in parotid granule lysates remained in the supernatant after sedimentation of granule membranes by centrifugation, suggesting that the activity is a soluble component of the secretion granule content.

Evidence of an Additional Subcellular Location of Peptide Amidation Activity. In addition to confirming its location in secretion granules, Fig. 1 shows that amidation activity is also detected at a level distinct from that of intact (arrow) or lysed (top of gradient) granules. This minor peak (highest in fraction 7: 0.7 M sucrose/5% Ficoll) appears in the region of endoplasmic reticulum and Golgi enzyme markers and represents <15% of the activity detected in the granule peak.

Biochemical Characteristics of the Parotid Granule Peptide Amidation Activity. The parotid granule peptide α-amidation enzyme is very similar to the enzyme(s) characterized in pituitary secretion granules with respect to several biochemical characteristics.

Copper ascorbate dependence. Addition of 3.5–6 μM $CuSO_4$ caused maximal stimulation of activity (3- to 4-fold). Activity decreased at higher copper concentrations such that half-maximal activity was found at ≈50 μM $CuSO_4$. Addition of Ca^{2+} or Mg^{2+} up to 1 mM had little effect on activity. Chelators of divalent cations, 2 μM diethyldithiocarbamic

Table 1. Peptide α-amidation activity measured in parotid granule fractions and secretion

Parotid preparation	Method	Peptide amidation/amylase, fmol·hr^{-1}·(unit of amylase)$^{-1}$
Secretion granules	Differential centrifugation	2.5 ± 0.9 (n = 5)
	Percoll gradient	2.1 ± 1.1 (n = 2)
Secretion	Cannulation *in situ*	4.2 ± 1.8 (n = 2)

Peptide α-amidation activity was measured in duplicate with 50–120 μg of secretory protein in 0.4 μM D-Tyr-Val-Gly/0.4 mM L-ascorbic acid/0.4 mg of catalase per ml/50 mM sodium Mops, pH 7.0. $CuSO_4$ concentration was adjusted to maximize activity measured in each sample. Amidation activity was normalized to amylase activity, since Percoll interferes slightly in the protein assay. The specific activity of α-amylase, measured in granule fractions depleted in Percoll by additional centrifugation or prepared in the absence of Percoll, was 2300 ± 470 units/mg of protein in all cases. Peptide amidation/α-amylase activity ratios were determined from n preparations, each measured in duplicate. Although comparable in magnitude, the level of amidation activity detected in cannulated secretion is somewhat higher than in purified secretion granule lysates. This is presently unexplained, although the possibility of an inhibitor in the secretion granule fractions, as has been described in pituitary granules (12), cannot be ruled out.

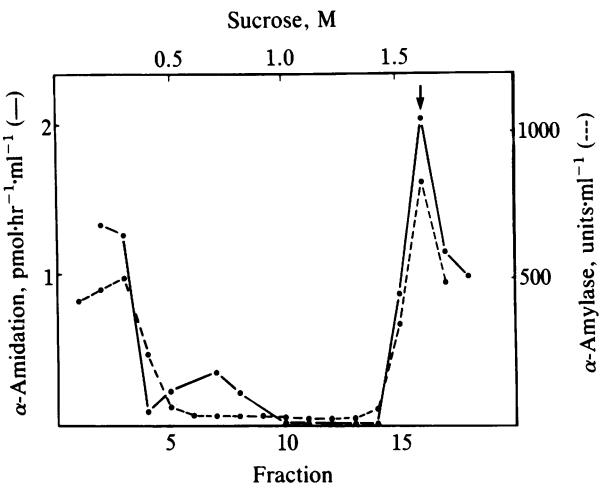

FIG. 1. Copurification of peptide α-amidation activity with parotid secretion granules. A parotid postnuclear supernatant was fractionated on a continuous sucrose density gradient (0.3 M sucrose in fractions 1–3 and a linear gradient of 0.3–2.0 M sucrose in fractions 3–20) containing 5% Ficoll 400, 2 mM sodium imidazole, and 0.5 mM EDTA (pH 7.0). Aliquots (0.5 ml) of 1-ml gradient fractions were dialyzed and concentrated prior to amidation assay, and the activities measured were normalized to the original fraction volume as described. Peptide α-amidation assays contained 0.7 μM D-Tyr-Val-Gly, 10 μM $CuSO_4$, 0.7 mM L-ascorbic acid, 0.8 mg of catalase per ml, and 50 mM sodium 3-(*N*-morpholino)propanesulfonate (sodium Mops) (pH 7.0). Peptide α-amidation activity (—) parallels α-amylase activity (---) throughout the secretion granule peak (arrow, maximum at 1.6 M sucrose).

acid (Et_2NCS_2H) or 300 μM EDTA (or EGTA), caused 95% inhibition of parotid activity. Addition of specific divalent cations (10 μM) in excess of Et_2NCS_2H (2 μM) revealed that only Cu^{2+} restored activity (Fig. 2). Similarly, inhibition caused by EDTA or EGTA was reversed by Cu^{2+} addition.¶

The parotid granule peptide amidation activity is stimulated by L-ascorbate (Fig. 2); maximal (3- to 5-fold) stimulation was found after addition of 0.3–1 mM L-ascorbate.

pH optimum. The pH dependence of parotid activity was measured between pH 5.5 and pH 8.0 in 50 mM sodium 2-(*N*-morpholino)ethanesulfonate/60 mM sodium Mops/0.5 mM L-ascorbate/0.3 mg of catalase per ml/5 μM $CuSO_4$/0.5–2 μM D-Tyr-Val-Gly; maximum activity was found between pH 6.5 and pH 7.0.

Enzyme kinetics. The parotid activity conforms to Michaelis–Menten kinetics; ascorbate increases substantially both the V_{max} and K_m (Fig. 3).

Exocytotic Discharge of Peptide α-Amidation Activity from Parotid Acinar Cells. Isoproterenol, a β-adrenergic agonist that stimulates selectively exocytosis of parotid granules (32), was used both *in vivo* and *in vitro* to demonstrate that peptide α-amidation activity is discharged from parotid acinar cells at the apical (luminal) surface.

(*i*) To study depletion *in vivo*, we measured peptide amidation and α-amylase specific activities in homogenates prepared from parotid glands removed from rats 60 min after intraperitoneal injection of isoproterenol (6 mg/150 g of body wt). Homogenates of stimulated glands (n = 3) contained

¶Peptide amidation activity in parotid granule fractions prepared by all three procedures exhibited similar biochemical properties except for Cu^{2+} dependence. EDTA addition minimized organelle aggregation and thus improved granule purity in both density gradient preparations. Granules isolated by these methods, unless dialyzed or washed repeatedly prior to assay, required high concentrations of added $CuSO_4$ (50–100 μM) for maximum activity. Granule fractions obtained by differential centrifugation (no EDTA added) required only 3.5–6 μM Cu^{2+}.

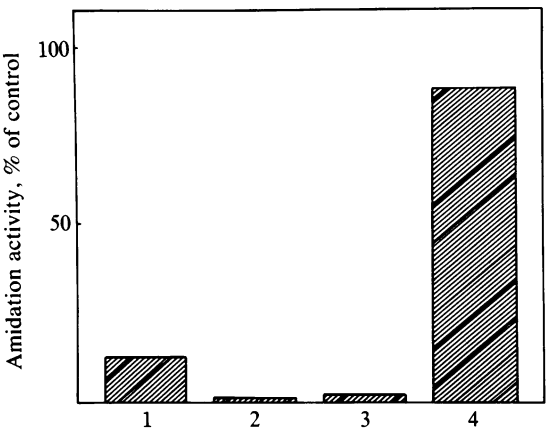

FIG. 2. Dependence of parotid granule peptide amidation activity on L-ascorbic acid and Cu^{2+}. Control activity was measured in parotid secretion granule lysates (60 μg of protein per assay) in 1.7 μM D-Tyr-Val-Gly/4 μM $CuSO_4$/0.8 mM L-ascorbic acid/0.5 mg of catalase per ml/50 mM sodium Mops, pH 7.0. Graph bars indicate the peptide α-amidation activity, expressed as a percentage of control activity, assayed in parallel with the following variations from control conditions: 1, absence of added L-ascorbic acid; 2, absence of added $CuSO_4$ and addition of 2 μM Et_2NCS_2H; 3, absence of added $CuSO_4$, addition of 2 μM Et_2NCS_2H, and 10 μM $CaCl_2$, $FeSO_4$, $Mg(NO_3)_2$, $MnCl_2$, or $Zn(OAc)_2$; 4, addition of 2 μM Et_2NCS_2H and 10 μM $CuSO_4$. Control reaction velocity was 9.6 pmol·hr^{-1}·(mg of protein)$^{-1}$.

<5% of the α-amylase specific activity measured in control homogenates (n = 3), and peptide α-amidation activity (maximized by 50–100 μM $CuSO_4$) was also depleted to a similar extent (<15% of control).

(*ii*) Parotid secretion was collected *in situ* by cannulation of the parotid duct of rats that had been injected with isoproterenol. This apically discharged secretion is essentially identical in composition to secretion granule content (20) and contains a similar level of peptide amidation activity (Table 1).

(*iii*) Although acinar secretory cells comprise the majority (>85% by volume; ref. 32) of cells in the parotid, a minority of other cell types border the ducts distal to the acinar clusters. To test whether the peptide α-amidation enzyme detected in the apical secretion originates from acinar cell discharge, we measured release from isolated parotid acini *in*

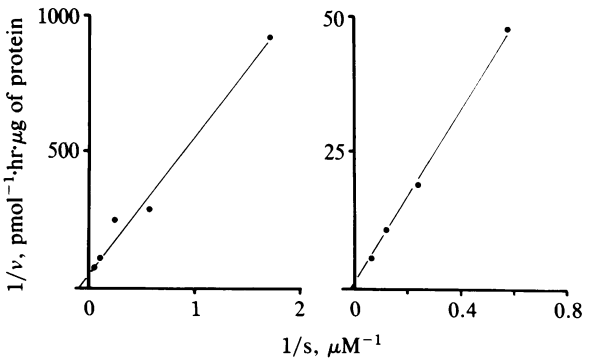

FIG. 3. Double reciprocal plots of peptide α-amidation kinetics measured in parotid secretion granule lysates. Peptide amidation activity was assayed in parotid granule lysates (80 μg of protein) with various concentrations of D-Tyr-Val-Gly (0.6–17 μM), 5 μM $CuSO_4$, and 50 mM sodium Mops (pH 7.0), in the absence (*Left*) or presence (*Right*) of 0.8 mM L-ascorbic acid and 0.5 mg of catalase per ml. Duplicate determinations of reaction velocity (v) agreed within 5% of their mean. Lines represent linear least-squares fits to the double reciprocal data, from which kinetic parameters were obtained. (*Left*) K_m = 9.3 μM, V_{max} = 0.019 pmol·hr^{-1}·(μg of protein)$^{-1}$. (*Right*) K_m = 80 μM, V_{max} = 1.0 pmol·hr^{-1}·(μg of protein)$^{-1}$. s, Substrate.

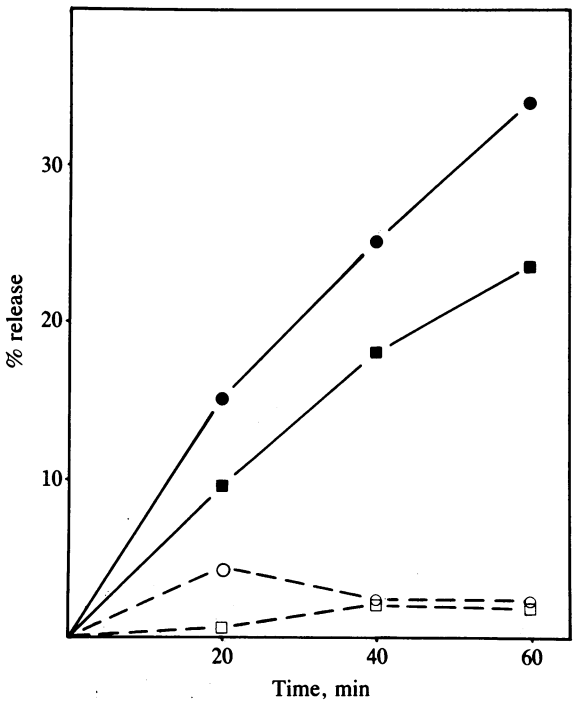

FIG. 4. Parallel secretion of peptide α-amidation and α-amylase activities from parotid acini *in vitro*. Parotid acini (≈100 mg wet weight in 10 ml of medium) were incubated in the presence (●, ■) or absence (○, □) of 20 μM isoproterenol as described. At the indicated time points, aliquots of medium were removed for peptide amidation (●, ○) or α-amylase (■, □) determination. At $t = 0$ an equal wet weight of unstimulated acini was homogenized in 10 ml of medium, and enzyme activities were measured in this crude homogenate. Enzyme activities discharged into the medium upon isoproterenol stimulation are given as a percentage of the whole-homogenate activities. Peptide amidation activity was assayed (in duplicate) with 0.2 μM D-Tyr-Val-Gly/10 μM $CuSO_4$/1 mM L-ascorbic acid/0.4 mg of catalase per ml/20 mM sodium Mops, pH 7.0. Cell integrity, measured by release of lactate dehydrogenase into the medium, was >93% throughout the incubations.

vitro; these acini release peptide amidation activity in parallel with α-amylase (Fig. 4).

Comparison of Relative Activity Levels in Parotid and Various Neural and Endocrine Secretion Granules. Because our kinetic analyses were carried out under conditions very similar to those used by others for the pituitary enzymes (12, 14), we can compare specific activities by examining appropriate V_{max} measurements. Accordingly, we estimate that parotid granules contain 3–4% of the specific activity of anterior pituitary granules and ≈35% as much as granules from either the intermediate or neural lobes.

Presence of Peptide α-Amidation Activity in Other Exocrine Granule Fractions. Purified secretion granule fractions from rat pancreas, submandibular, and lacrimal glands were found to contain a level of amidation activity readily detectable by the radiochemical assay (Table 2); limited characterization indicates stimulation of activity by ascorbate and dependence on Cu^{2+} concentration.

Table 2. Peptide α-amidation activity measured in exocrine secretion granules purified from various rat tissues

Source of granule fraction	Peptide amidation activity, pmol·hr^{-1}·(mg of protein)$^{-1}$
Parotid gland	6.3
Pancreas	6.1
Submandibular gland	1.7
Lacrimal gland	4.6

Peptide amidation activity was determined in parallel by incubation of granule lysates (100 μg of protein per assay) with 0.7 μM D-Tyr-Val-Gly/0.7 mM L-ascorbic acid/0.6 mg of catalase per ml/50 mM sodium Mops, pH 7.0. $CuSO_4$ concentration was adjusted to maximize activity in each sample. Activities presented are the mean of duplicate determinations (duplicates varied <5% from their mean).

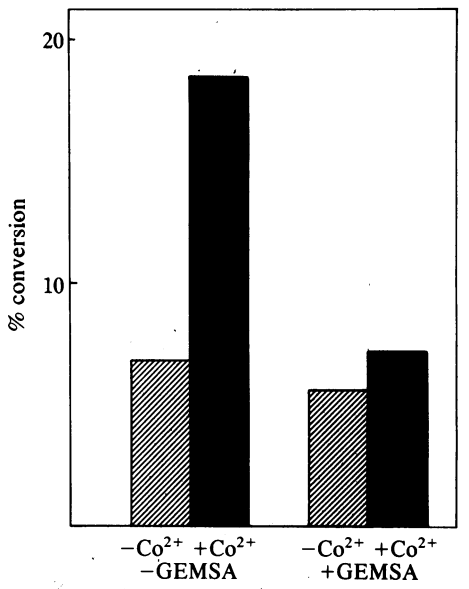

FIG. 5. B-type carboxypeptidase activity in parotid granule lysates. Freeze–thaw lysates of parotid granule fractions (prepared by differential centrifugation; 10 μg of protein) were preincubated (60 min at 0–4°C) in the absence or presence of 1 mM $CoCl_2$ or 1 mM guanidinoethylmercaptosuccinic acid (GEMSA) (or both, as indicated) in 75 mM sodium acetate (pH 5.6). Enzyme incubation (60 min at 37°C) was initiated by addition of 50 μM BzPhe-Ala-Arg containing 100,000 cpm of tritiated BzPhe-Ala-Arg, and carboxypeptidase activity was determined by chloroform extraction.

Evidence of a B-Type Carboxypeptidase Activity in Exocrine Secretion Granules. Neural and endocrine tissues contain a granule-associated B-type carboxypeptidase that is thought to produce glycine-terminated substrates for conversion to peptide amides (1). Parotid granule lysates contain an activity that resembles the B-type carboxypeptidase(s) contained in neural and endocrine granules (33, 34) with respect to apparent pH optimum (≈pH 6 in the presence of 50 μM substrate), stimulation by Co^{2+} and inhibition by guanidinoethylmercaptosuccinic acid (Fig. 5). Although further characterization is clearly required, it is unlikely that this activity represents lysosomal contamination of the parotid granule fraction, since the lysosomal carboxypeptidase B is not Co^{2+}-stimulated (25).

DISCUSSION

In this study we report that exocrine secretion granules from rat parotid acinar cells contain and discharge a peptide α-amidation enzyme. Purified granule fractions, prepared by several different procedures, contain comparable amounts of peptide α-amidation activity. Examination of the secretory behavior of the activity confirms that the enzyme is present in the granule content of acinar cells and indicates that peptide amidation enzyme is secreted at the apical epithelial surface by exocytosis, in parallel with α-amylase.

Partial characterization of the parotid peptide α-amidation activity reveals strong similarities to the enzyme present in pituitary secretion granules and indicates that parotid and other exocrine granules contain a significant, although lower, amount of activity relative to neural and endocrine granules that package amidated peptides.

It has been known for some time that parotid granules contain L-ascorbic acid, but no physiologic role of this molecule is known in exocrine granules. The present finding of an ascorbate-dependent peptide amidation enzyme in parotid and other exocrine secretion granules suggests that intragranular ascorbate may function as a cofactor for α-amidation of exocrine secretory polypeptides. Although well-established in neural and endocrine systems, the possibility of intragranular processing in exocrine cells is novel. In neural and endocrine granules, peptide α-amidation occurs in concert with other post-translational modifications [e.g., proteolysis and acetylation (1)]. Therefore, we have begun to search for the presence in exocrine secretion granules of other enzymes known to be involved in intragranular processing reactions. One such enzyme, a B-type carboxypeptidase thought to function in biosynthesis of enkephalins and other neuroendocrine peptides (25), can be identified on the basis of its pH optimum, divalent-cation dependence, and inhibitor sensitivity. Parotid granule fractions contain a similar activity, suggesting that exocrine secretion granules, like neural and endocrine secretion granules, may be capable of conducting a number of post-translational modification reactions in their interior.

Previous studies of exocrine secretory proteins have focused on relatively large (>30 kDa) major species. In neural and endocrine systems, however, intragranular processing occurs primarily at the level of smaller species. We have been focusing on eight polypeptides with apparent mass ranging from 10 to 25 kDa (by $NaDodSO_4$/PAGE) as potential candidates for involvement in processing reactions during secretion granule maturation. Because α-amidated peptides described to date are considerably smaller (<10 kDa) than these molecules, it may be necessary to resort to other analytical approaches (e.g., refs. 2–4). We note in this regard that several peptides of mass <10 kDa have been purified from parotid saliva (35–37), and it has been found recently that salivary gland extracts contain small as-yet-uncharacterized peptide(s) that react with antisera against helodermin, the carboxyl-terminally α-amidated venom peptide (38). In summary, although specific amidated peptides have not been identified as yet, the present finding of processing machinery whose only known function is in the intragranular modification of secretory polypeptides forms a rational basis for viewing exocrine granules not merely as repositories for secretory products but as potential sites for ongoing post-translational processing concomitant with packaging operations.

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1. Mains, R. E., Eipper, B. A., Glembotski, C. C. & Dores, R. M. (1983) *Trends Neurosci.* **6**, 229–235.
2. Tatemoto, K. & Mutt, V. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4115–4119.
3. Tatemoto, K. & Mutt, V. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6603–6607.
4. Tatemoto, K., Carlquist, M. & Mutt, V. (1982) *Nature (London)* **296**, 659–660.
5. Uvnas-Wallensten, K. (1976) *Acta Physiol. Scand. Suppl.* **438**, 1–39.
6. Takeuchi, T., Takemoto, T., Tani, T. & Miwa, T. (1973) *Lancet* **ii**, 920.
7. Sandrin, E. & Boissonnas, R. A. (1962) *Experientia* **18**, 58–61.
8. Bernardi, L., Bosisio, G., Goffredo, O. & deCastiglione, R. (1964) *Experientia* **20**, 489–492.
9. Kreil, G., Mollay, C., Kaschnitz, R., Maiml, L. & Vilas, U. (1980) *Ann. N.Y. Acad. Sci.* **343**, 338–345.
10. Krieger, D. T. (1983) *Science* **222**, 975–985.
11. Bradbury, A. F., Finnie, M. D. A. & Smyth, D. G. (1982) *Nature (London)* **298**, 686–688.
12. Eipper, B. A., Mains, R. E. & Glembotski, C. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5144–5148.
13. Glembotski, C. C. (1984) *J. Biol. Chem.* **259**, 13041–13048.
14. Glembotski, C. C., Eipper, B. A. & Mains, R. E. (1984) *J. Biol. Chem.* **259**, 6385–6392.
15. Thorn, N. A. & Christensen, B. L. (1984) *Acta Physiol. Scand.* **121**, 47A (abstr.).
16. Russell, J. T., Levine, M. & Njus, D. (1985) *J. Biol. Chem.* **260**, 226–231.
17. Kizer, J. S., Busby, W. H., Cottle, C. & Youngblood, W. W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3228–3232.
18. von Zastrow, M., Tritton, T. R. & Castle, J. D. (1984) *J. Biol. Chem.* **259**, 11746–11750.
19. von Zastrow, M., Tritton, T. R. & Castle, J. D. (1984) *J. Cell Biol.* **99**, 353 (abstr.).
20. Cameron, R. S. & Castle, J. D. (1984) *J. Membr. Biol.* **79**, 127–144.
21. Schramm, M. & Danon, D. (1961) *Biochim. Biophys. Acta* **50**, 102–112.
22. Arvan, P., Rudnick, G. & Castle, J. D. (1984) *J. Biol. Chem.* **259**, 13567–13572.
23. Herzog, V. & Farquhar, M. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5073–5077.
24. Eipper, B. A., Glembotski, C. C. & Mains, R. E. (1983) *Peptides* **4**, 921–928.
25. Fricker, L. D. & Snyder, S. (1983) *J. Biol. Chem.* **258**, 10950–10955.
26. Stack, G., Fricker, L. & Snyder, S. (1984) *Life Sci.* **34**, 113–121.
27. Mains, R. E. & Eipper, B. A. (1984) *Endocrinology* **115**, 1683–1690.
28. Bernfeld, P. (1955) *Methods Enzymol.* **1**, 149–158.
29. Schnaar, R. L., Weigel, P. H., Kuhlenschmidt, M. S., Lee, Y. C. & Roseman, S. (1978) *J. Biol. Chem.* **253**, 7940–7951.
30. Udenfriend, S., Stein, S., Bohlen, P., Dairman, W., Leimgruber, W. & Weigele, M. (1972) *Science* **178**, 871–872.
31. Tanaka, T., Gresik, E. W. & Barka, T. (1981) *J. Histochem. Cytochem.* **29**, 1189–1195.
32. Schramm, M. & Selinger, Z. (1974) *Adv. Cytopharmacol.* **2**, 29–32.
33. Hook, V. Y. H. & Loh, Y. P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2776–2780.
34. Fricker, L. D., Supattapone, S. & Snyder, S. H. (1982) *Life Sci.* **31**, 1841–1844.
35. Saitoh, E., Isemura, S. & Sanada, K. (1983) *J. Biochem. (Tokyo)* **93**, 495–502.
36. Saitoh, E., Isemura, S. & Sanada, K. (1983) *J. Biochem. (Tokyo)* **93**, 883–888.
37. Holbrook, I. B. & Molan, P. (1975) *Biochem. J.* **149**, 489–492.
38. Robberecht, P., Vandermeers, A., Vandermeers-Piret, M. C., Svoboda, M., DeNeef, P., DeGraef, J., Woussen-Colle, M. C., Yanaihara, N., Yanaihara, C. & Christophe, J. (1985) *Regulatory Peptides* Suppl. 3, S17 (abstr.).