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Carboxypeptidase B-like converting enzyme activity in secretory granules of rat pituitary

(vasopressin/melanotropin/adrenocorticotropin/neuropeptide biosynthesis/prohormone processing)

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ABSTRACT **Recent amino acid sequence data suggest that trypsin-like and carboxypeptidase B-like activities are required for the processing of pituitary prohormones—e.g., pro-opiocortin (pro-adrenocorticotropin/lipotropin) and provasopressin in secretory granules. In this study the existence of a carboxypeptidase B activity in purified secretory granules from anterior, intermediate, and neural lobes of rat pituitary has been examined. A carboxypeptidase B activity that cleaved the COOH-terminal -Lys-Lys-Arg residues from the adrenocorticotropin fragment ACTH-(1–17) (a potential hormone product liberated from pro-opiocortin by a trypsin-like enzyme) was detected in anterior and intermediate lobe granules. A similar carboxypeptidase B activity was also present in purified secretory granules from rat pituitary neural lobes that cleaved the -Lys-Arg residues from [Arg^8]vasopressin-Gly-Lys-Arg, a potential product cleaved from provasopressin. Secretory granule carboxypeptidase(s) from the three lobes of the pituitary was shown to cleave ^{125}I-[Met]enkephalin-Arg^6 to form ^{125}I-[Met]enkephalin as well. ^{125}I-[Met]Enkephalin was used as a model substrate for the quantitative assay of pituitary carboxypeptidase activity. The carboxypeptidase B in secretory granules from all three lobes was shown to be active at pH 5.5, but not at pH 7.4. Inhibition by the zinc metallocarboxypeptidase inhibitors guanidinopropylsuccinic acid, aminomercaptosuccinic acid, benzylsuccinic acid, 2-mercaptomethyl-3-guanidinoethylthiopropanoic acid, and the potato carboxypeptidase B inhibitor, and inhibition by the metal chelators EDTA and 1,10-phenanthroline demonstrate metal ion dependence of the pituitary granule carboxypeptidase activities. However, Co^{2+} stimulated the secretory granule carboxypeptidase B activities. Thiol protease inhibitors such as Cu^{2+} and *p*-chloromercuriphenylsulfonic acid also inhibited the activity. Thus, the secretory granule carboxypeptidase B-like activities in all three lobes of the pituitary appear to be similar thiol-metallopeptidases that differ from other carboxypeptidase activities previously described and may play an exclusive role in hormone biosynthesis in the pituitary.**

Studies on the biosynthesis of peptide hormones have shown that they are initially synthesized as part of larger inactive polypeptide prohormones. Limited proteolysis of the prohormone must occur to yield the biologically active hormone. Within the cell, the prohormone is first synthesized at the rough endoplasmic reticulum (RER) and then inserted into the RER cisternae, similar to other proteins destined for secretion (1). It is subsequently routed to the Golgi apparatus, where it is packaged into secretory granules (2–4). Limited proteolysis of the prohormone is thought to occur within the granule (2–4), which later undergoes exocytosis to release the active hormone products to the extracellular environment.

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Analysis of the amino acid sequences of the prohormone precursors for adrenocorticotropin/lipotropin (ACTH/LPH; pro-opiocortin) (5), [Arg^8]vasopressin (provasopressin) (6), and others (7–10) have revealed that multiple hormones and peptides may be cleaved from these precursors. The hormone(s) or peptide sequences within the precursor are characteristically flanked at their NH_2 and COOH termini by pairs of basic amino acids (lysine, arginine). It has, therefore, been proposed that a multi-step process requiring different proteases may be involved in processing. Initially, a trypsin-like enzyme that cleaves either between or on the carboxyl terminal side of the paired basic amino acid residues of the prohormone could liberate peptide hormone(s) extended at the carboxyl terminus with lysine or arginine. A carboxypeptidase B-like enzyme could then cleave the COOH-terminal lysine and arginine residues from these peptides to yield the final hormones. If the initial cleavage occurs between the paired basic residues, an aminopeptidase may also be involved to remove the NH_2-terminal basic residue extension from the cleaved peptides.

Thus far, little is known about the prohormone proteolytic processing enzymes. Studies on such enzymes would give a better understanding about how a cell regulates the specific cleavage of a large prohormone to produce biologically active peptide product(s). On the basis of the hypothesis that trypsin-like and carboxypeptidase B-like activities are required for prohormone processing, we have begun to search for such enzymes in secretory granules, where processing is thought to occur. Recent studies have demonstrated the existence of a unique "trypsin-like" protease in the secretory granules of rat and bovine pituitary anterior and intermediate lobes. This enzyme appears to be specific for the paired basic residues of the prohormone, pro-opiocortin, synthesized by these lobes (11–13). Likewise, a similar enzyme activity, found in bovine neural lobe secretory granules, cleaves the endogenous prohormone, provasopressin (ref. 12 and unpublished data).

In this study, we report the presence of a carboxypeptidase B-like activity in anterior and intermediate lobe secretory granules that cleaved the COOH-terminal -Lys-Lys-Arg basic residues from ACTH-(1–17) to produce ACTH-(1–14). A carboxypeptidase B-like activity was also found in neural lobe secretory granules that cleaved the Lys-Arg residues from [Arg^8]vasopressin-Gly-Lys-Arg (AVP-GLA). These carboxypeptidase activities were shown to be different from other known carboxypeptidases and may serve to remove the COOH-terminal basic residue(s) from the peptide prod-

Abbreviations: ACTH, adrenocorticotropin; LPH, lipotropin; α-MSH, α-melanotropin; AVP, [Arg^8]vasopressin; AVP-GLA, AVP-Gly-Lys-Arg (the shorter homologs are abbreviated similarly); GPSA, guanidinopropylsuccinic acid; APMSA, aminomercaptosuccinic acid; BzlSA, benzylsuccinic acid; MGTA, 2-mercaptomethyl-3-guanidinoethylthiopropanoic acid; PCMPSA, *p*-chloromercuriphenylsulfonic acid.

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ucts cleaved from the prohormones by the trypsin-like enzymes.

METHODS AND MATERIALS

Preparation of Secretory Granules from Anterior, Intermediate, and Neural Lobes. Female rats (Osborne–Mendel strain, weighing 250–300 g) were obtained from the National Institutes of Health animal facility. Anterior, intermediate, and neural lobes from rat pituitaries were dissected and homogenized separately in 2 ml of 0.25 M sucrose/10 mM Tris·HCl, pH 7.4, at 0°C, using a Potter–Elvehjem homogenizer. The homogenate was then subjected to differential centrifugation, as described previously (11–13), to obtain crude granule-enriched fractions (P_{III} fraction). The P_{III} fraction was further purified by discontinuous sucrose density centrifugation (11–13), and 15 25-drop fractions were collected. Fractions were analyzed for ACTH (anterior lobe), α-melanotropin (α-MSH) (intermediate lobe), or [Arg^8]vasopressin (AVP) (neural lobe), β-glucuronidase (lysosomal enzyme marker), and monoamine oxidase (mitochrondrial enzyme marker) by methods described previously (14, 15). Fraction 6 (neural and intermediate lobes) or fraction 5 (anterior lobe) was highly enriched in secretory granules (11–13). Granules were lysed by freezing and thawing six times to liberate and expose the prohormone-processing enzymes to added substrates.

Incubation of Lysed Secretory Granules with ACTH-(1–17) (Anterior and Intermediate Lobes) or AVP-GLA (Neural Lobe). An aliquot of secretory granule lysate from anterior or intermediate lobes of rat pituitary (5–15 μg of protein) was incubated with 25 μg of ACTH-(1–17) (Bachem, Torrance, CA), 50 mM potassium phosphate buffer, pH 5.5, and 100 mM NaCl in a total volume of 30–40 μl at 37°C for 1 or 2 hr. The reaction was stopped by placing the samples on ice and adding 30 μl of 10% (wt/vol) acetic acid in 95% (vol/vol) EtOH. After Vortex mixing and sitting on ice for 15–30 min, samples were centrifuged for 2 min in a Beckman Microfuge. The supernatant was removed and immediately subjected to high-voltage electrophoresis.

Crude AVP-GLA (approximately 85% pure, Peninsula Laboratories, San Carlos, CA) was purified by reverse-phase HPLC on an LC18 column (Supelco, Bellefonte, PA). Peak fractions of AVP-GLA (eluting at approximately 19–20% acetonitrile in 0.25 M triethylamine/formic acid, pH 2.5) were dried overnight in a vacuum centrifuge. The peptide was resuspended in 50 mM potassium phosphate buffer, pH 5.5, and incubated with granule lysate from neural lobe, under the same conditions as described above for anterior and intermediate lobes. Samples were then subjected to high-voltage electrophoresis.

When ACTH-(1–17) or AVP-GLA was incubated with pancreatic carboxypeptidase B (Sigma), potassium phosphate buffer of pH 7.4 instead of pH 5.5 was used.

High-Voltage Electrophoresis. Samples (5–10 μl) in 10% acetic and 90% ethanol were spotted on cellulose thin-layer chromatography plates (Analtech, Newark, DE). The high-voltage electrophoresis buffer contained formic acid/acetic acid/water (20/80/900, vol/vol), pH 2, and electrophoresis was at 900 V until the methyl green marker had migrated 13 cm from the origin. Peptides were visualized by the fluorescamine method (16).

Carboxypeptidase Assay for the Conversion of ^{125}I-[Met]-Enkephalin-Arg^6 to ^{125}I-[Met]Enkephalin. [Met]Enkephalin-Arg^6 (Peninsula Laboratories) was iodinated by the chloramine-T method (17) and the carboxypeptidase assay was carried out as described (18). ^{125}I-[Met]Enkephalin-Arg^6 and ^{125}I-[Met]enkephalin in the reaction mixture were separated by thin-layer chromatography on silica gel G plates (Analtech) in a solvent system of pyridine/ethyl acetate/water/acetic acid, (33/53/9.4/4.3, vol/vol). Radioactivity was scanned along each lane with the use of a BID System 100 radiogram imaging system (Bioscan, Washington, DC).

RESULTS

Secretory Granule Preparations. To determine the purity of the secretory granule preparations, monoamine oxidase (a mitochondrial marker enzyme) and β-glucuronidase (a lysosomal marker enzyme) were measured in the secretory granule fractions from rat pituitary anterior, intermediate, and neural lobes. Less than 1% of total tissue MAO or β-glucuronidase was found in each purified granule fraction (Table 1). Because only the most pure fractions of granules from each lobe were used, the recovery of total tissue granules was small as reflected by the low percent of total tissue hormone content in each granule fraction (Table 1).

Carboxypeptidase Activity in Lysed Pituitary Granules. Carboxypeptidase activity was demonstrated in lysed pituitary granules from anterior and intermediate lobes by following the conversion of ACTH-(1–17) to ACTH-(1–14). The sequential removal of the three COOH-terminal basic residues from ACTH-(1–17) should result in the formation of ACTH-(1–16), ACTH-(1–15), and ACTH-(1–14). On the basis of their charge differences, it should be possible to separate these peptides by high-voltage electrophoresis. Incubation of ACTH-(1–17) with purified pancreatic carboxypeptidase B (2 μg) resulted in the formation of three fluorescamine-labeled spots, which migrated with standard ACTH-(1–14), lysine, and arginine (Fig. 1, lane 6). Pancreatic carboxypeptidase B at a lower concentration (1 μg) converted ACTH-(1–17) to two products which migrated at intermediate positions between standard ACTH-(1–17) and ACTH-(1–14) (Fig. 1, lane 5). Because sequential cleavage of the -Lys-Lys-Arg residues from ACTH-(1–17) should result in peptides with progressively less charge, ACTH-(1–16) and ACTH-(1–15) would be expected to migrate between ACTH-(1–17) and ACTH-(1–14) during high-voltage electrophoresis. Thus, the three peptides formed from ACTH-(1–17) by pancreatic carboxypeptidase B (1 μg) are most likely to be ACTH-(1–16), ACTH-(1–15), and ACTH-(1–14).

Incubation of ACTH-(1–17) with lysed secretory granules from rat anterior pituitary resulted in the formation of peptide products that migrated with those formed by pancreatic carboxypeptidase B (Fig. 1). When ACTH-(1–17) was incubated for 1 hr with a 2-μl sample of lysed anterior pituitary granules, two fluorescamine-labeled spots that migrated with ACTH-(1–17) and ACTH-(1–16) were formed (Fig. 1, lane 7). When ACTH-(1–17) was incubated with a larger amount of enzyme (10 μl), three fluorescamine-labeled spots were present that migrated with ACTH-(1–16), ACTH-(1–15), and ACTH-(1–14) standards (Fig. 1, lane 8).

Incubation of ACTH-(1–17) with lysed secretory granules from intermediate lobe (10 μl) resulted in the formation of a fluorescamine-labeled spot that migrated with ACTH-(1–15) and ACTH-(1–14) (Fig. 1, lane 9). Although this spot was not

Table 1. Characterization of purified pituitary secretory granule fractions

Source of purified granules	% total tissue enzyme or hormone in granule fraction		
	Monoamine oxidase	β-Glucuronidase	Hormone*
Anterior lobe	0.2	0.3	16
Intermediate lobe	0	0.2	5.8
Neural lobe	0	0.8	4.2

Monoamine oxidase is a marker enzyme for mitochondria; β-glucuronidase is a marker enzyme for lysosomes.
*Hormones measured in anterior, intermediate, and neural lobes are ACTH, α-MSH, and AVP, respectively.

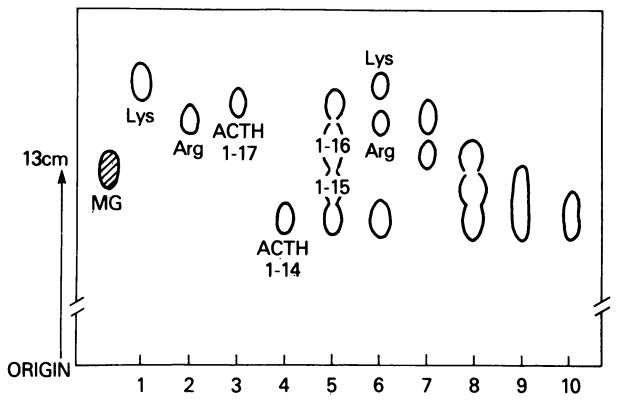

FIG. 1. Cleavage of ACTH-(1–17) by carboxypeptidase activities in rat pituitary anterior and intermediate lobe secretory granules. ACTH-(1–17) and cleavage products were separated by high-voltage electrophoresis on cellulose thin-layer chromatography plates. The migration position of standards are shown: lane 1, lysine; lane 2, arginine; lane 3, ACTH-(1–17); lane 4, ACTH-(1–14). The results of incubation of ACTH-(1–17) with enzyme samples are shown: lane 5, 1 μg of pancreatic carboxypeptidase B incubated for 60 min; lane 6, 2 μg of pancreatic carboxypeptidase B incubated for 60 min; lane 7, 2 μl of lysed anterior lobe secretory granules incubated for 60 min; lane 8, 10 μl of lysed anterior lobe secretory granules incubated for 60 min; lane 9, 10 μl of lysed intermediate lobe secretory granules incubated for 120 min; and lane 10, 30 μl of lysed intermediate lobe secretory granules incubated for 120 min. MG indicates the migration position of the marker methyl green.

clearly seen as two separate products, its migration with ACTH-(1–15) and ACTH-(1–14) suggests that it may be composed of these two peptides. When ACTH-(1–17) was incubated with a greater amount of lysed intermediate lobe secretory granules, a spot migrating at the position of ACTH-(1–14) was formed (Fig. 1, lane 10). No fluorescamine-labeled spots were observed when intermediate and anterior lobe granule lysates (10 μl) were incubated without ACTH-

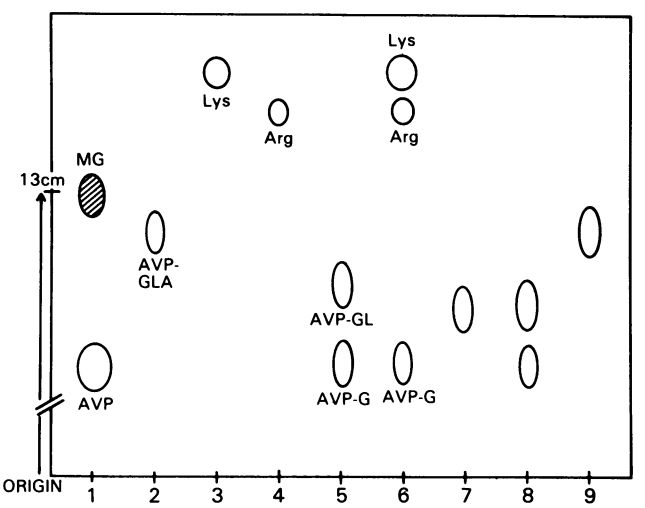

FIG. 2. Cleavage of AVP-GLA by carboxypeptidase activities in rat pituitary neural lobe secretory granules. AVP-GLA and cleavage products were separated by high-voltage electrophoresis on cellulose thin-layer chromatography plates. The migration positions of standards are shown; lane 1, AVP; lane 2, AVP-GLA; lane 3, lysine; lane 4, arginine. The results of incubation of AVP-GLA with enzyme samples are shown; lane 5, 1 μg of pancreatic carboxypeptidase B incubated for 60 min; lane 6, 2 μg of pancreatic carboxypeptidase B incubated for 60 min; lane 7, 2 μl of lysed neural lobe secretory granules incubated for 2 hr; lane 8, 10 μl of neural lobe secretory granules incubated for 2 hr; lane 9, 5 μl of boiled sample of neural lobe granules incubated for 2 hr. MG indicates the migration position of the marker methyl green.

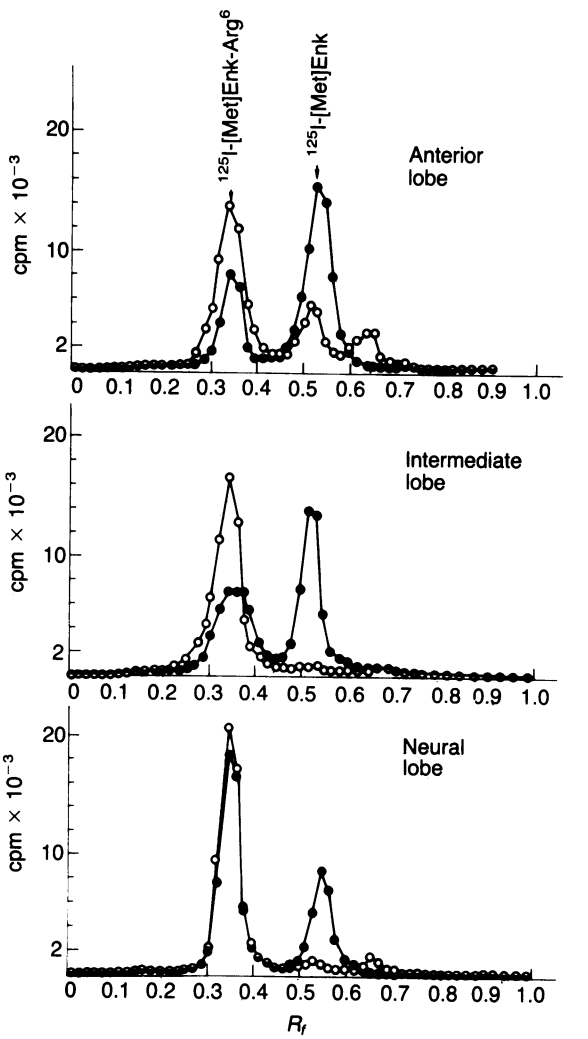

FIG. 3. Carboxypeptidase activity in secretory granules from rat pituitary. Chromatogram showing the substrate and product formed after incubation (30 min, 37°C) of secretory granule lysates from anterior lobe, intermediate lobe, and neural lobe with ^{125}I-[Met]enkephalin-Arg^6 at pH 5.5 (●—●) or pH 7.5 (○—○). The mobilities of the substrate ^{125}I-[Met]enkephalin-Arg^6 and the product ^{125}I-[Met]enkephalin are indicated by the arrows.

(1–17). ACTH-(1–17) was not degraded when incubated with boiled granule lysate from anterior and intermediate lobe (data not shown).

Conversion of ACTH-(1–17) to ACTH-(1–14) by lysed anterior and intermediate lobe granules was also confirmed by reverse-phase HPLC separation of the radiolabeled substrate ^{125}I-ACTH-(1–17) and radiolabeled product ^{125}I-ACTH-(1–14) (data not shown).

Carboxypeptidase activity in neural lobe secretory granules was demonstrated by following the removal of COOH-terminal basic residues from AVP-GLA (Fig. 2). AVP-GL and AVP-G, predicted products of carboxypeptidase B cleavage of AVP-GLA, should be less charged than the substrate AVP-GLA. These peptides should be separable by high-voltage electrophoresis. Purified pancreatic carboxypeptidase B converted AVP-GLA to two new fluorescamine-labeled products identified as AVP-GL and AVP-G on the basis of their charge differences (Fig. 2, lane 5). Incubation with a larger amount of pancreatic carboxypeptidase B resulted in a spot corresponding to AVP-G and two spots that ran with the amino acids lysine and arginine (Fig. 2, lane 6). Lysed neural lobe secretory granules (2 μl) converted AVP-GLA to a product that migrated with one produced by

pancreatic carboxypeptidase B, AVP-GL (Fig. 2, lane 7). Incubation of AVP-GLA with a larger sample of neural lobe granules (10 μl) produced two spots that migrated with AVP-GL and AVP-G (Fig. 2, lane 8). It is noted that AVP standard and AVP-G (formed from AVP-GLA by pancreatic carboxypeptidase) migrate to approximately the same position. Because the secretory granules should contain amidation enzyme(s) that can convert AVP-G to AVP (19), the products formed by incubation of AVP-GLA with aliquots of lysed neural lobe granules may include, in addition to AVP-GL and AVP-G, some AVP. AVP-GLA was not degraded when incubated with boiled neural lobe granule lysate, and AVP-GLA incubated without enzyme migrated to the same position as standard AVP-GLA (data not shown). No fluorescamine-labeled spots were seen when neural lobe granule lysate (10 μl) was incubated without AVP-GLA.

Characterization of Pituitary Granule Carboxypeptidase B Activity. Characterization of carboxypeptidase activities requires a quantitative enzyme assay. Because the high-voltage electrophoresis method cannot adequately quantitate the amount of peptide present and because of the instability of the iodinated ACTH peptide fragments, further study of the carboxypeptidases in pituitary secretory granules was performed by following the conversion of a model substrate ^{125}I-[Met]enkephalin-Arg^6 to ^{125}I-[Met]enkephalin.

Incubation of anterior lobe, intermediate lobe, and neural lobe granule lysates with ^{125}I-[Met]enkephalin-Arg^6 at pH 5.5 resulted in the appearance of a peak of radioactivity that chromatographed with ^{125}I-[Met]enkephalin (Fig. 3). In addition, ^{125}I-[Met]enkephalin-Lys^6 was also converted to ^{125}I-[Met]enkephalin (data not shown). No carboxypeptidase activity in any of the granule fractions was found at pH 7.5.

To determine whether the carboxypeptidase activity in secretory granules of rat pituitary is similar to other known carboxypeptidases, the effect of peptidase inhibitors was examined (Table 2). Inhibitors of zinc metallopeptidases GPSA, APMSA, BzlSA, MGTA (20–22), and the carboxypeptidase inhibitor from potatoes (23) inhibited carboxypeptidase B activity in secretory granule lysates from anterior, intermediate, and neural lobes. The metal chelators EDTA and 1,10-phenanthroline also inhibited carboxypeptidase activity from all three lobes of rat pituitary. Cd^{2+} inhibited activity in granule lysates from all three lobes of the pituitary. Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} had differential effects on carboxypeptidase activity in granule lysates; however, their effects were not large. Co^{2+} dramatically stimulated carboxypeptidase activity in granule lysates from the three lobes. The degree of Co^{2+} stimulation is probably much greater than indicated since it was evident that the substrate, ^{125}I-[Met]enkephalin-Arg^6, became depleted (data not shown).

PCMPSA and Cu^{2+}, reagents that react with thiol groups (24), inhibited carboxypeptidase activity in granules from anterior and neural lobes and had a small effect on that from intermediate lobe. The reducing agents dithiothreitol and mercaptoethanol had no effect on carboxypeptidase activity from anterior and neural lobe granules, but they slightly inhibited activity from intermediate lobe granules. Leupeptin (25) also inhibited activity in granules from the three lobes. Serine protease inhibitors such as phenylmethylsulfonyl fluoride (26) had little or no effect on the carboxypeptidase activity.

Table 2. Effect of protease inhibitors and divalent cations on pituitary secretory granule and lysosomal carboxypeptidases

Compound	Conc., μM	Carboxypeptidase activity, % of control					
		Granules			Lysosomes		
		AL	IL	NL	AL	IL	NL
None		100	100	100	100	100	100
Zn metallopeptidase inhibitors							
Potato inhibitor	46	17	12	27	103	118	120
GPSA	640	0	0	6	23	28	33
APMSA	490	0	0	0	24	37	42
BzlSA	460	4	0	3	93	97	133
MGTA	590	0	0	0			
Metal ion chelators							
1,10-Phenanthroline	140	10	5	7	23	36	64
EDTA	140	20	15	21	25	47	65
Thiol protease inhibitors							
PCMPSA	14	27	33	39	23	26	48
$CuCl_2$	140	30	71	5	140	43	101
Divalent cations							
$CdCl_2$	100	39	42	42	36	29	64
$CoCl_2$	1400	205	141	190	90	40	31
$CaCl_2$	100	95	96	94	65	39	59
$MgCl_2$	100	103	84	98	38	23	79
$MnCl_2$	100	95	70	88	54	29	49
$NiCl_2$	100	104	91	81	29	46	66
$PbCl_2$	100	103	82	90	38	41	84
ZnOAc	140	110	85	96			
Others							
Dithiothreitol	1400	117	77	76			
Leupeptin	2.9	0	0	0			
Phenylmethylsulfonyl fluoride	140	65	86	97	29	25	49

Values are averages from two or three samples. AL, anterior lobe; IL, intermediate lobe; NL, neural lobe; GPSA, guanidinopropylsuccinic acid; APMSA, aminomercaptosuccinic acid; BzlSA, benzylsuccinic acid; MGTA, 2-mercaptomethyl-3-guanidinoethylthiopropanoic acid; PCMPSA, *p*-chloromercuriphenylsulfonic acid.

To differentiate the carboxypeptidase in granules from that in lysosomes, the effect of protease inhibitors and divalent cations on lysosomal carboxypeptidase(s) was studied (Table 2). Fraction 11 from the discontinuous sucrose density gradient of P_{III} (refs. 11 and 13 and *Methods and Materials*) possessed the greatest amount of β-glucuronidase activity (lysosomal marker) and was used to examine lysosomal carboxypeptidase activity. The potato carboxypeptidase inhibitor and BzlSA inhibited granular carboxypeptidase but had no effect on lysosomal carboxypeptidase. The cations Ca^{2+}, Mg^{2+}, Mn^{2+}, and Ni^{2+} inhibited lysosomal carboxypeptidase activity but had no effect on the activity in granules. Co^{2+} had no effect on lysosome activity but stimulated the activity in granules. Phenylmethylsulfonyl fluoride inhibited lysosomal carboxypeptidase more than that in secretory granules. Like the granular carboxypeptidase, the lysosomal enzyme activities were inhibited by the metal ion chelators EDTA and 1,10-phenanthroline and the thiol protease inhibitors PCMPSA and Cu^{2+}.

DISCUSSION

Several criteria should be met for a peptidase to be relevant in the processing of a particular hormone. The processing enzyme should be present in granules where hormone processing is thought to occur, it should function at the intragranular pH, and, finally, it should cleave the endogenous hormone precursor appropriately. Carboxypeptidase activity has been demonstrated in pituitary secretory granules and was active at pH 5.5, consistent with the intragranular pH of 5.0–5.8 (27–29).

The peptides ACTH-(1–17) and AVP-GLA are expected products resulting from the cleavage of pro-opiocortin and provasopressin, respectively, by the trypsin-like converting enzyme (11–13). Hence, these two peptides were chosen as representative endogenous substrates for examining the specificity of the pituitary granule carboxypeptidase. The carboxypeptidase activity in secretory granule lysates of anterior and intermediate lobes, which synthesize pro-opiocortin, sequentially cleaved the COOH-terminal -Lys-Lys-Arg residues of ACTH-(1–17) to form ACTH-(1–14). Carboxypeptidase activity from secretory granules of neural lobe cleaved Lys-Arg residues from AVP-GLA. Thus, the carboxypeptidase B-like activity detected in pituitary secretory granules fulfills the criteria outlined and may be involved in the processing of the respective prohormones synthesized by each lobe.

Characterization of carboxypeptidase activity, measured by following the conversion of ^{125}I-[Met]enkephalin-Arg^6 to ^{125}I-[Met]enkephalin, showed that the enzyme in secretory granules from all three lobes of the rat pituitary appears to be similar. The granular carboxypeptidase from each lobe was inhibited by the potato carboxypeptidase B inhibitor, GPSA, APMSA, BzlSA, and MGTA, which typically inhibit zinc metalloenzymes (20–23). The enzyme activities in granules from the three pituitary lobes were all dramatically stimulated by Co^{2+} and inhibited by Cd^{2+}. Inhibition by EDTA and 1,10-phenanthroline suggest that the secretory granule carboxypeptidases may be metalloenzymes. The carboxypeptidases were also inhibited by Cu^{2+} and PCMPSA, indicating that they may be thiol proteases, unlike pancreatic carboxypeptidase B. Furthermore, the differential effects of protease inhibitors and cations on carboxypeptidase activity from secretory granules and lysosomes show that the granular carboxypeptidase activity demonstrated here is distinct from that localized in lysosomes.

In conclusion, rat anterior, intermediate, and neural lobe secretory granules appear to have a similar thiol-metallopeptidase that exhibits carboxypeptidase B specificity for basic residues at the COOH-terminus of peptide hormones. The similar effects of many protease inhibitors on carboxypeptidase activity in pituitary granules, in bovine adrenal medulla chromaffin granules (refs. 18 and 30–32; unpublished data), and in rat islets of Langerhans (33) suggest that perhaps a unique, universal carboxypeptidase B-like enzyme exists that is specifically involved in the processing of peptide hormones.

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