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Activation and Membrane Binding of Carboxypeptidase E

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Carboxypeptidase E (CPE)is a carboxypeptidase B-like enzyme that is thought to be involved in the processing of peptide hormones and neurotransmitters. Soluble and membrane-associated forms of CPE have been observed in purified secretory granules from various hormone-producing tissues. In this report, the influence of membrane association on CPE activity has been examined. A substantial amount of the membrane-associated CPE activity is solubilized upon extraction of bovine pituitary membranes with either 100 mM sodium acetate buffer (pH 5.6) containing 0.5% Triton X-100 and 1 M NaCl, or by extraction with high pH buffers (pH > 8). These treatments also lead to a two- to threefold increase in CPE activity. CPE extracted from membranes with either NaCl/Triton X-100 or high pH buffers hydrolyzes the dansyl-Phe-Ala-Arg substrate with a lower K_m than the membrane-associated CPE. The V_{max} of CPE present in extracts and membrane fractions after the NaCl/Triton X-100 treatment is twofold higher than in untreated membranes. Treatment of membranes with high pH buffers does not affect the V_{max} of CPE in the soluble and particulate fractions. Pretreatment of membranes with bromoacetyl-D-arginine, an active site-directed irreversible inhibitor of CPE, blocks the activation by NaCl/Triton X-100 treatment. Thus the increase in CPE activity upon extraction from membranes is probably not because of the conversion of an inactive form to an active one, but is the result of changes in the conformation of the enzyme that effect the catalytic activity.

Key words: enkephalin convertase, carboxypeptidase H, carboxypeptidase B-like, neuropeptide biosynthesis

Many peptide hormones and neurotransmitters are initially produced as large precursors that must be enzymatically processed into the bioactive peptides [1]. The processing sites are usually pairs of basic amino acids, and the sequential action of a trypsin-like endopeptidase and a carboxypeptidase B-like exopeptidase produces the bioactive peptides. Carboxypeptidase E (CPE, EC 3.4.17.10, also designated enkephalin convertase and carboxypeptidase H) is a carboxypeptidase B-like enzyme associated with the biosynthesis of numerous peptide hormones and neurotransmitters. CPE is present in many tissues where peptide biosynthesis occurs, such as brain, pituitary,

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and adrenal medulla [2–8]. Within the pituitary, adrenal medulla, and an insulin-producing tumor, CPE is associated with the peptide hormone-containing secretory granule fraction [3,6–8], which is the putative site of peptide processing. CPE is maximally active at pH 5.6 [3,6], the intragranular pH of pituitary and adrenal medulla secretory granules [9,10].

Within the secretory granules, CPE is present in several forms. One form is soluble upon extraction of lysed granules with low ionic strength buffers, such as 10 mM sodium acetate, pH 6 [3]. A small amount of the membrane-associated CPE activity is solubilized by 1 M NaCl [11]. Some of the remaining membrane-associated CPE activity is solubilized with buffers containing both 1 M NaCl and 0.5% Triton X-100 in 10 mM sodium acetate, pH 6 [12]. The different forms of CPE have been purified to apparent homogeneity using affinity and ion exchange chromatography [12,13]. Both the soluble and membrane associated forms of CPE have similar enzymatic and physical properties [3,12,13]. The only detectable difference between the purified forms of CPE is the apparent molecular weight on SDS polyacrylamide gels, with the soluble form (50,000 daltons) slightly smaller than the form extracted from membranes by NaCl/Triton X-100 (52,000 daltons). Both forms have the same amino acid sequence of the N-terminal region [14].

Recently a cDNA clone encoding bovine CPE has been isolated and sequenced [14]. Southern blot analysis of bovine genomic DNA indicates that a single gene encodes CPE. Northern blot analysis of bovine pituitary mRNA shows several species that hybridize with the cDNA probes [14]. One of these species accounts for $> 95\%$ of the CPE mRNA, and the other RNA species presumably arises from alternate polyadenylation sites within the 3′ untranslated region [14]. These results suggest that the differences between the soluble and membrane forms of CPE are the result of post-translational modifications of a single precursor protein. This protein does not contain hydrophobic regions that would be predicted to form transmembrane-spanning domains. Instead, a potential amphipathic helix located within the C-terminal region may assist in the association with membranes [14,15], although there is no direct evidence for this possibility.

Antisera raised against the purified soluble form of CPE binds to both soluble and membrane-extracted forms with equal affinity [16]. When examined by either radioimmunoassay or immunoblot techniques, comparable amounts of CPE immunoreactivity are present in soluble and membrane fractions of adrenal medulla chromaffin granule lysates [17,18]. However, when similar fractions are examined for carboxypeptidase activity, there is significantly more CPE activity in the soluble extracts [3,17]. This finding suggests that the specific activity of the soluble form is greater than the specific activity of the membrane-bound form. Since CPE purified to apparent homogeneity from either soluble or membrane extracts has a similar specific activity [12], it is likely that the membrane form is less catalytically active when bound to membranes. In the present study, this possibility has been tested by measuring the enzymatic activity and kinetic parameters of the membrane form of CPE both before and after solubilization, using different extraction conditions.

MATERIALS AND METHODS

Frozen bovine pituitaries were purchased from Pelfreeze. Membranes were prepared by homogenizing (Brinkman Polytron) the pituitaries in 5 volumes 100 mM

NaAc, pH 5.6. The homogenate was centrifuged for 1 hour at 150,000*g*. The supernatant was removed, and the pellet was resuspended in 100 mM NaAc, pH 5.6, and centrifuged at 150,000*g* for 1 hour. After two extractions with 100 mM NaAc, the pellet was similarly extracted twice with 1 M NaCl in 100 mM NaAc buffer, pH 5.6. The pellet was resuspended in H_2O, frozen (−20°C), thawed, and centrifuged as above. The supernatant was removed, and the pellet was resuspended in 10 mM NaAc buffer, pH 5.6. This pellet is referred to as the "membrane" fraction.

Carboxypeptidase E activity was assayed using dansyl-Phe-Ala-Arg, as previously described [13]. In a typical assay, tissue extract, NaAc buffer, pH 5.6 (50–100 mM final concentration), and substrate (100 μM final concentration) were combined in a total volume of 250 μl. The samples were incubated at 37°C for 20 min, and the reaction was stopped with 100 μl 0.5 M HCl. Chloroform (2 ml) was added, the tubes were mixed and centrifuged (1 minute at 1,000 rpm in a Beckman TJ-6), and the fluorescence in the lower organic phase was determined in a Perkin-Elmer LS-3 fluorimeter. Substrate is insoluble in chloroform, whereas the product (dansyl-Phe-Ala) is highly soluble in this solvent [13]. Standard curves using dansyl-Phe-Ala were used to convert the fluorimeter readings into nmoles product. All determinations were performed in triplicate. Carboxypeptidase E activity was calculated from the difference between enzymatic activity measured in the presence and absence of 1 μM GEMSA. These conditions have been previously shown to be specific for CPE [12,19,20]. In some cases, the CPE activity has been adjusted for the number of pituitary glands used in the membrane preparation, which allows the results of separate experiments to be compared.

RESULTS

As previously reported [3,11,12], bovine pituitary glands contain several forms of CPE activity. One of these activities is observed in the supernatant of pituitaries extracted with 10 mM NaAc, pH 5.6 (Fig. 1). Repeated homogenization and extraction with either 10 or 100 mM NaAc, pH 5.6, does not extract the majority of the membrane-associated enzymatic activity. High salt buffers (1 M NaCl/100 mM NaAc, pH 5.6) extract some of the membrane-associated activity. The addition of 0.5% Triton X-100 to the high salt buffer solubilizes a substantial amount of the membrane-bound activity. However, some CPE activity remains associated with the membranes even after a second extraction with 0.5% Triton X-100 in high salt buffer (Fig. 1).

To investigate whether solubilization of the membrane-associated form leads to an increase in the enzymatic activity, pituitary membranes were prepared by repeated homogenization and extraction of bovine pituitaries with 100 mM NaAc, pH 5.6, and then 1 M NaCl in the same buffer. Treatment of these washed membranes with 0.5% Triton X-100 in the high salt buffer, followed by centrifugation, solubilizes a substantial amount of the CPE activity (Fig. 2). Quantitation of this enzymatic activity shows there is more activity in the supernatant than is present in the membranes before treatment with NaCl/Triton X-100. The level of CPE activity in the membranes after extraction with NaCl/Triton X-100 is only slightly lower than the level of activity present in the homogenate before the extraction. The net result is a two- to threefold increase in the total CPE activity upon NaCl/Triton X-100 treatment of membranes, with 65% of the CPE activity solubilized by the treatment. Centrifugation of untreated

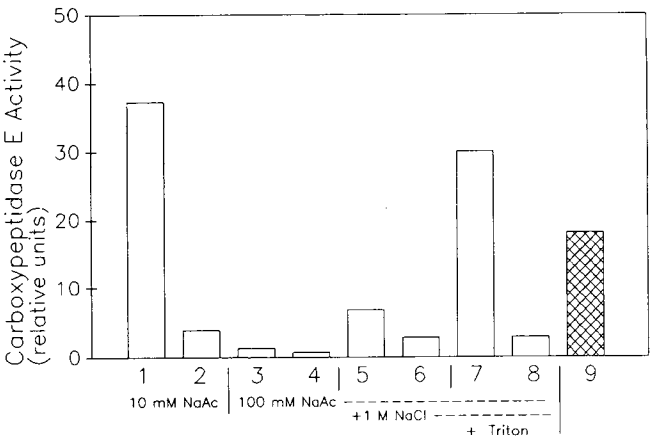

Fig. 1. Extraction of CPE activity from bovine pituitary. One whole pituitary was homogenized (Polytron) in 10 ml of 10 mM NaAc buffer, pH 5.6, and then centrifuged at 50,000*g* for 10 min. The supernatant was removed **(column 1)** and the pellet resuspended in the same buffer. The homogenate was centrifuged as above and the supernatant removed **(column 2)**. The pellet was extracted in a similar manner with 100 mM NaAc, pH 5.6 **(columns 3, 4)**, with 1 M NaCl in 100 mM NaAc, pH 5.6 **(columns 5, 6)**, and then with 0.5% Triton X-100 in the 1 M NaCl/100 mM NaAc buffer, pH 5.6 **(columns 7, 8)**. The pellet was resuspended in 10 ml 100 mM NaAc, pH 5.6 **(column 9)**, and aliquots of each fraction were assayed for CPE activity as described in Materials and Methods.

membrane homogenates does not produce an increase in CPE activity, and all of the enzymatic activity is recovered in the membrane fraction.

Treatment of bovine pituitary membranes with high pH buffers (100 mM $NaHCO_3$, pH 9) also leads to a solubilization and elevation of the CPE activity (Fig. 2). As with the NaCl/Triton X-100 extraction, the level of CPE activity in the supernatant following the high pH treatment is greater than the level of activity in the *untreated* membranes. The CPE activity remaining in the membranes is lower after extraction with $NaHCO_3$ than with NaCl/Triton X-100. The total CPE activity is approximately twofold higher after $NaHCO_3$ extraction, compared with the level in the membranes before extraction.

Treatment of pituitary membranes with buffers other than $NaHCO_3$ also leads to a solubilization of the CPE activity (Fig. 3). Sodium borate buffers, ranging from pH 8 to pH 10, are comparable to $NaHCO_3$ in their effect on the membrane association and activity of CPE. The increase in enzymatic activity in the soluble extracts is accompanied by a decrease in enzymatic activity remaining in the membranes. However, the sum of the extracted and membrane-bound activities is two- to threefold higher than when membranes are treated with NaAc, pH 5.6 (Fig. 3). Sodium acetate buffer at pH 6.0 is comparable to the same buffer at pH 5.6, with all of the enzymatic activity remaining membrane-bound. Interestingly, treatment of membranes with sodium phosphate buffers of pH 5.6 or 6.0 leads to a small increase in the activity present in the soluble extracts. Sodium phosphate buffers in the pH 6–8 range are more effective in solubilizing the membrane-bound activity than the lower pH buffers, but are not as effective as the sodium borate buffers. Tris-chloride buffers of pH 7.4–8.4 vary in effectiveness, with the lower pH buffer comparable to sodium phosphate and the higher pH buffers comparable to sodium borate and sodium

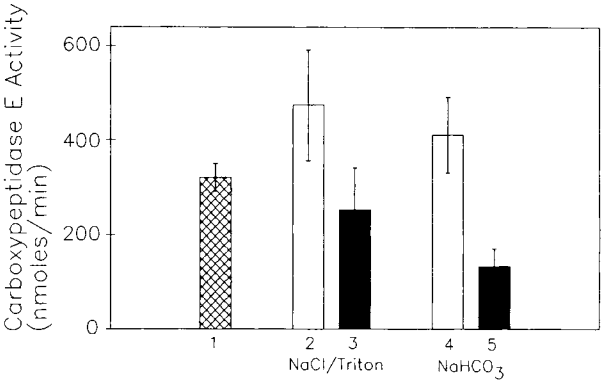

Fig. 2. Quantitation of CPE activity in membrane homogenates before and after extraction. Bovine pituitary membranes were prepared as described in Materials and Methods, using high-speed centrifugation (150,000*g* for 1 hour). After two extractions with 100 mM NaAc, pH 5.6, and two with 1 M NaCl in NaAc buffer, the membranes were resuspended in H_2O, frozen (−20°C), thawed, and centrifuged as above. The supernatant was removed and the pellets resuspended in 10 mM NaAc buffer, pH 5.6. An aliquot of this homogenate was removed for the carboxypeptidase assay **(column 1)**. The remainder was treated with an equal volume of either 200 mM $NaHCO_3$ or 1% Triton X-100/2 M NaCl/200 mM NaAc, pH 5.6. The samples were centrifuged as above, the supernatants removed, and the pellets resuspended in either 100 mM $NaHCO_3$ or 0.5% Triton X-100/1 M NaCl/50 mM NaAc, pH 5.6. After centrifugation, the supernatants were combined with the first extracts and the pellets resuspended in 100 mM NaAc, pH 5.6. Aliquots of the extracts **(columns 2, 4)** and the membranes after extraction **(columns 3, 5)** were assayed for carboxypeptidase activity after an appropriate dilution into 100 mM NaAc buffer, pH 5.6. Results of two separate experiments are shown, with the variation as indicated (error bars show standard deviation). CPE activity is expressed in nmol/min per pituitary gland used in the preparation of membranes.

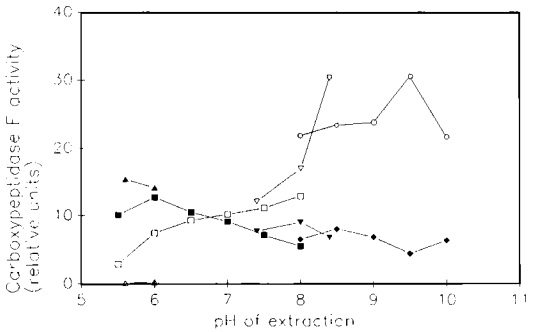

Fig. 3. Effect of pH on the association of CPE with membranes. Bovine pituitary membranes were prepared as described in Materials and Methods. Membrane homogenates were treated with various buffers (50 mM final concentration) as described for the $NaHCO_3$ treatment in the Figure 2 legend. After centrifugation (1 hour at 150,000*g*) the supernatants were removed and immediately diluted into 4 volumes of 380 mM NaAc, pH 5.6. Pellets were resuspended in 300 mM NaAc buffer, pH 5.6, and aliquots of both the supernatants **(open symbols)** and pellets **(filled symbols)** were assayed for carboxypeptidase activity as described in Materials and Methods. Buffers used were: sodium acetate **(triangles)**; sodium phosphate **(squares)**; Tris-chloride **(inverted triangles)**; sodium borate **(diamonds)**.

bicarbonate. Treatment of the membranes with buffers of pH > 10, such as sodium carbonate, leads to a total loss of CPE enzymatic activity (data not shown).

CPE enzymatic activities in the different extracts or membrane fractions are similarly inhibited by active site-directed inhibitors (Fig. 4), supporting the proposal that these are different forms of CPE. The small differences between fractions are not significant; the variation between fractions is comparable to the error of the carboxypeptidase assay for each fraction. Carboxypeptidase activity in all of the fractions assayed is substantially inhibited (> 90%) by 1 μM GEMSA, 10 μM MGTA, or 100 μM APMSA. Lower concentrations of the inhibitors show partial inhibition, with very little inhibition (< 10%) observed with 10 nM MGTA or 100 nM APMSA. These results indicate that in the fractions assayed, the predominant dansyl-Phe-Ala-Arg hydrolyzing carboxypeptidase activity (at pH 5.6) is CPE. When the initial 100 mM NaAc extracts of the pituitaries are analyzed in a similar manner, approximately 10–20% of the carboxypeptidase activity is not inhibited by high concentrations of the inhibitors (data not shown).

Kinetic analysis of the CPE activity in membrane homogenates shows a K_m of 45 μM and a V_{max} of 0.64 μmol/min per pituitary (Fig. 5A and Table I). After extraction of the membranes with NaCl/Triton X-100, the activity remaining in the membrane fraction shows a K_m of 43 μM and a V_{max} of 0.80 μmol/min per pituitary (Fig. 5B). The activity present in the soluble extracts has a K_m of 22 μM and a V_{max}

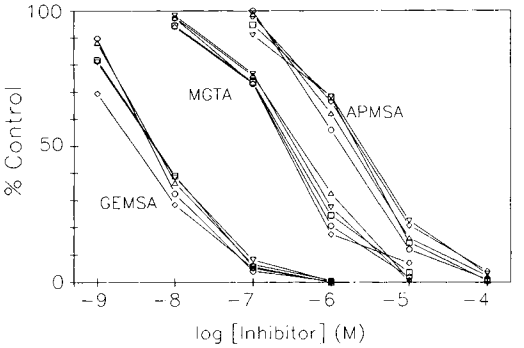

Fig. 4. Carboxypeptidase activity in bovine pituitary extracts or membrane homogenates vs. inhibitor concentration. Pituitary fractions were assayed for carboxypeptidase activity in the presence of various concentrations of inhibitors, as described in Materials and Methods, using a substrate concentration of 20 μM dansyl-Phe-Ala-Arg. Fractions used were 1 M NaCl/100 mM NaAc extracts (○), NaCl/Triton X-100 extracts (△), $NaHCO_3$ extracts (□), membranes after extraction with NaCl/Triton X-100 (▽), and membranes after extraction with $NaHCO_3$ (◇), prepared as described in the Figure 2 legend. GEMSA, guanidinoethylmercaptosuccinic acid; MGTA, 2-mercaptomethyl-3-guanidinothiopropanoic acid; APMSA, aminopropylmercaptosuccinic acid.

Fig. 5. Kinetic analysis of CPE activity in membrane homogenates and extracts. Bovine pituitary extracts and membranes were prepared as described in Materials and Methods and the Figure 2 legend. Fractions were diluted and assayed for carboxypeptidase activity as described in Materials and Methods, using substrate (dansyl-Phe-Ala-Arg) concentrations of 20, 28.6, 50, 100, and 200 μM. Fractions assayed were as follows: **A:** membrane homogenates before extraction with NaCl/Triton X-100 or $NaHCO_3$ (■); **B:** NaCl/Triton X-100 extracts (○) and membranes after extraction with NaCl/Triton X-100 (●); **C:** $NaHCO_3$ extracts (◇) and membranes after extraction with $NaHCO_3$ (◆). CPE activity is expressed in μmol/min per pituitary gland used in the membrane preparation.

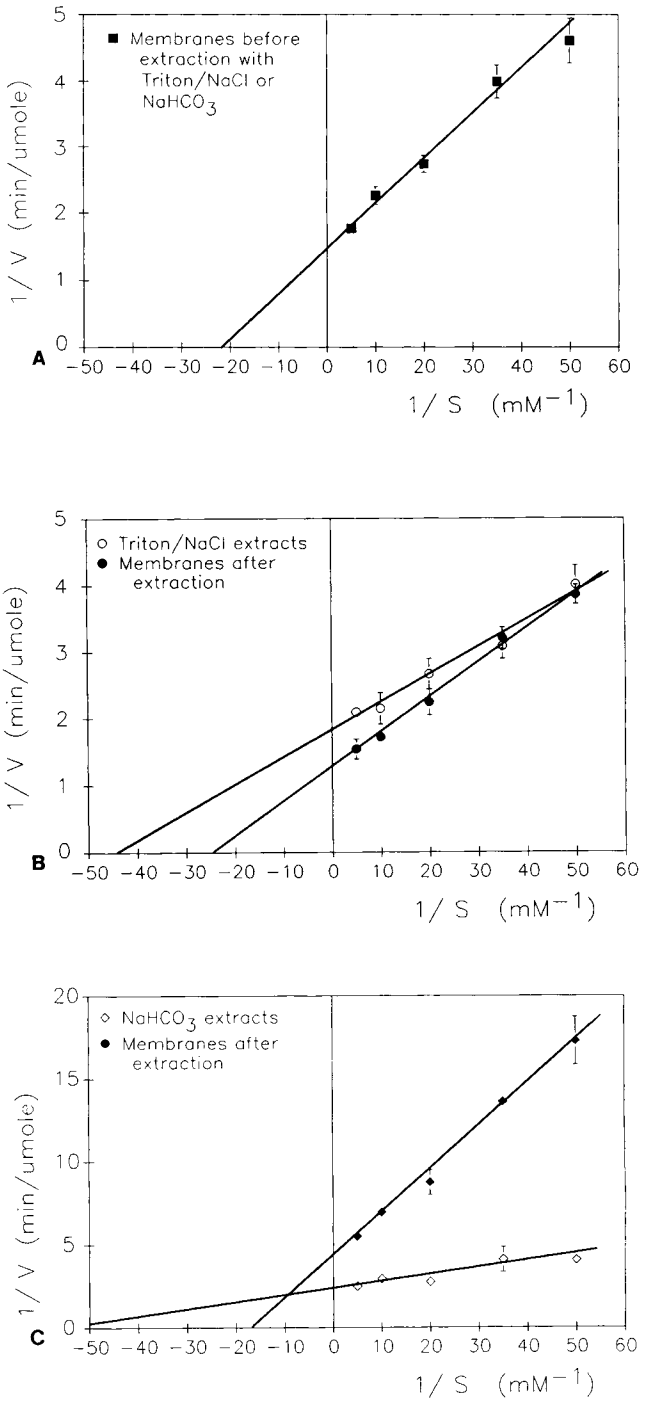
■ Membranes before extraction with Triton/NaCl or $NaHCO_3$
1/V (min/umole)
1/S (mM^{-1})
A
○ Triton/NaCl extracts
● Membranes after extraction
B
◇ $NaHCO_3$ extracts
● Membranes after extraction
C

Figure 5.

TABLE I. Kinetic Analysis of CPE in Bovine Pituitary Membranes, NaCl/Triton X-100 Extracts, and $NaHCO_3$ Extracts

Fraction	K_m (μM)	V_{max} (μmol/min)
Membranes before extraction	45 ± 10	0.64 ± 0.04
NaCl/Triton X-100 extraction	22 ± 4	0.56 ± 0.08
Membranes after NaCl/Triton X-100	43 ± 10	0.80 ± 0.04
Total after treatment		1.36 ± 0.09
$NaHCO_3$ extraction	18 ± 8	0.32 ± 0.04
Membranes after $NaHCO_3$	59 ± 12	0.22 ± 0.02
Total after treatment		0.54 ± 0.05

of 0.56 μmol/min. The total V_{max} for NaCl/Triton X-100 extracts and membranes after extraction is 1.36 μmol/min per pituitary, an increase of 110% compared with untreated membranes. Similar changes in the K_m for CPE are obtained when pituitary membranes are extracted with $NaHCO_3$ (Fig. 5C). Membranes after extraction show a K_m of 59 μM, whereas the soluble extracts show a K_m of 18 μM. However, the V_{max} does not increase upon extraction with $NaHCO_3$, with values of 0.32 and 0.22 μmol/min per pituitary for soluble extracts and membranes after extraction, respectively. The total V_{max} for $NaHCO_3$ extracts and membranes after extraction is 0.54 μmol/min per pituitary, which is not substantially different from the V_{max} for untreated membranes.

To investigate whether the increase in V_{max} for CPE upon treatment with NaCl/Triton X-100 is because of the conversion of an inactive enzyme into the active form, membranes were pretreated with an irreversible inhibitor of CPE. Bromoacetyl-D-arginine has previously been shown to irreversibly inactivate CPE in a concentration and time-dependent manner [20]. The inhibition can be blocked by substrate, suggesting that the inhibitor is binding to the active site of the enzyme [20]. When membranes are pretreated with bromoacetyl-D-arginine for 30 min at 37°C and then washed several times to remove unreacted inhibitor, there is a concentration-dependent loss of CPE activity (Fig. 6). With 0.1 mM bromoacetyl-D-arginine treatment, CPE enzymatic activity is reduced to 18% of the control level. Treatment with 1 mM bromoacetyl-D-arginine leads to a complete loss of CPE activity. The increase in CPE activity observed when membranes are treated with 0.5% Triton X-100 and 1 M NaCl is eliminated by either concentration of inhibitor (Fig. 6). This suggests that the increase in CPE activity upon NaCl/Triton X-100 treatment is not a result of the activation of an inactive membrane-bound form of CPE.

DISCUSSION

These experiments confirm the presence of multiple forms of CPE and demonstrate that CPE activity is influenced through an interaction with membranes. Several forms of CPE are present in bovine pituitary gland extracts, as previously reported [3,11,12]. The form of CPE that remains membrane-bound after extraction with NaCl/Triton X-100 has not been previously described and was probably overlooked, since repeated extractions were not routinely performed [12]. Alternatively, the assay conditions are not identical, which could affect the results. Previous studies used dansyl-Phe-Leu-Arg as substrate, and compared the activity in the presence and

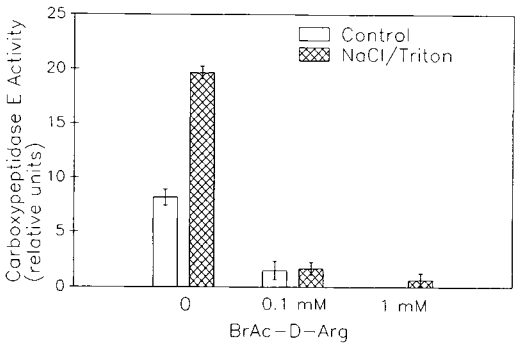

Fig. 6. Effect of bromoacetyl-D-arginine pretreatment on carboxypeptidase E activity in bovine pituitary membranes. Membranes were incubated with the indicated concentration of bromoacetyl-D-arginine in 100 mM NaAc buffer, pH 5.6, for 30 min at 37°C. Ten volumes of 100 mM NaAc, pH 5.6, were added and the samples centrifuged (10 min at 50,000*g*). The pellets were resuspended in 100 mM NaAc buffer, pH 5.6, and centrifuged as above. These pellets were resuspended in 100 mM NaAc buffer and treated either with H_2O or with Triton X-100 and NaCl to give final concentrations of 0.5% and 1 M, respectively. Aliquots were assayed for CPE activity as described in Materials and Methods.

absence of 1 mM $CoCl_2$. The present study used dansyl-Phe-Ala-Arg, which is hydrolyzed by purified CPE with a higher K_{cat} and lower K_m than dansyl-Phe-Leu-Arg, and defined CPE activity as the difference in carboxypeptidase activity measured in the presence and absence of 1 μM GEMSA. These conditions are more selective for CPE and provide a better estimate of CPE activity [19].

The finding that the enzymatic activity in the different fractions is potently inhibited by several active site-directed inhibitors supports the conclusion that these activities are different forms of CPE, rather than different enzymes. These inhibitors have been used to differentiate CPE from other carboxypeptidase B-like enzymes, such as carboxypeptidase B (CPB) and carboxypeptidase N (CPN). The IC_{50} of these inhibitors for unpurified CPE activity in the various bovine pituitary fractions is similar to their K_i values for purified CPE, which is different from their K_i values for other enzymes. GEMSA, APMSA, and MGTA inhibit purified CPE with K_i values of 8, 390, and 180 nM, respectively [20]. In contrast, MGTA is 20–100 times more potent as an inhibitor of CPN than CPE [21], while GEMSA and APMSA are several hundred-fold less potent inhibitors of CPB and CPN than CPE [22,23]. Other carboxypeptidase B-like activities, such as the lysosomal carboxypeptidase B, are not affected by these inhibitors [20]. Thus it appears likely that the carboxypeptidase activities in the different extracts of bovine pituitary glands are due to different forms of CPE. This proposal is supported by studies examining CPE immunoreactivity in different fractions of chromaffin granule lysates [17,18]. Immunoreactive CPE is found in low and high ionic strength extracts, and in membranes after the extractions [17].

The solubilization of some of the membrane-associated CPE activity by extraction with neutral or high pH buffers is consistent with previous studies examining carboxypeptidase activities in rat and anglerfish pancreatic tissue [6,24,25]. A CPE-like enzyme has been purified and characterized from a rat pancreatic tumor by Docherty and Hutton [6,24]. They found very little membrane-bound CPE activity upon extraction of the tissue with 25 mM Tris-chloride, pH 8 [24]. Since this was the

only buffer used in their study, it was not clear if this difference was a result of the extraction conditions or was due to tissue-specific modifications of CPE that affect membrane binding. A CPE-like enzyme has been described in anglerfish pancreatic islet secretory granules by Mackin and Noe [25]. They find that this CPE-like enzyme is membrane-bound when extracted at pH 5.2 and soluble when extracted at pH 7.4. The absence of a soluble form upon extraction at low pH may be a difference between species. When a variety of species were examined for CPE-like activity, only mammalian tissues were found to contain substantial amounts of the form of CPE that is soluble at pH 5.6 [26]. A similar CPE-like enzyme was found in shark, *Xenopus*, and *Aplysia* neural tissue, with the majority of the activity membrane-bound upon extraction at low pH [26]. The reason for this species difference is not known. The influence of membrane binding on enzymatic activity was not addressed in these studies.

Analysis of the kinetic parameters for substrate (dansyl-Phe-Ala-Arg) hydrolysis shows the solubilized CPE to have a lower K_m than the membrane-associated form (Fig. 5). Extraction with NaCl/Triton X-100 leads to a twofold increase in V_{max}, whereas extraction with $NaHCO_3$ does not significantly effect the V_{max}. The most plausible interpretation of these results is that the interaction of CPE with a membrane component influences the enzymatic activity. An alternative interpretation is that Triton X-100 and/or NaCl directly affects CPE, causing an increase in the V_{max} along with a decrease in the K_m for dansyl-Phe-Ala-Arg hydrolysis. However, neither Triton X-100 or NaCl appear to activate purified CPE (unpublished observation). Also, high pH extraction of CPE from membranes leads to an activation of enzymatic activity in the absence of Triton X-100 and NaCl. This finding supports the proposal that membrane-associated CPE is less catalytically active than the soluble form [17]. It is unlikely that a membrane-associated form is totally inactive, since pretreatment of membranes with bromoacetyl-D-arginine blocks the NaCl/Triton X-100 activation of CPE enzymatic activity.

The physiological significance of these findings is not understood. Since the endoplasmic reticulum and cis Golgi compartments are thought to have a neutral internal pH, CPE would presumably exist as a soluble protein in these organelles. As secretory granules form and mature, the internal pH becomes more acidic, with a final pH of approximately 5.5 for bovine pituitary secretory granules [9], which should lead to an association of one of the forms of CPE with the secretory granule membranes. When the secretory granules fuse with the cellular membrane, the pH would increase to that of the extracellular environment, which is typically in the neutral pH range, and would lead to a solubilization and activation of some of the membrane-associated activity. However, this activation would be countered by a decrease in CPE activity at pH values above pH 5.6, the optimum pH for enzymatic activity.

The molecular basis of the different forms of CPE is not known. The finding that CPE can be extracted from bovine pituitary membranes by neutral pH buffers is consistent with the proposal that an amphipathic helix within the C-terminal region of CPE helps anchor the protein to the membranes [14,15]. However, these results do not rule out other possible mechanisms for the membrane binding, such as ionic interactions with membrane-bound proteins and/or hydrophobic post-translational modifications of CPE. It is unlikely that a trans-membrane spanning domain serves as the membrane anchor, since this should not be a pH-dependent interaction. Furthermore, no potential trans-membrane spanning domains are found within the

amino acid sequence of CPE [14,15]. Further studies examining the molecular mechanism of the membrane interaction are necessary.

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