The proteinase mPC1, a neuroendocrine member of the mammalian family of subtilisin-like enzymes, has previously been shown to be converted to a carboxy-terminally truncated 66-kDa form during transport through the secretory pathway. The cleavage site and the function of this carboxyl-terminal truncation event are unknown. We have performed site-directed mutagenesis of two paired basic sites in the mPC1 carboxy-terminal tail and expressed these constructs in PC12 cells, a rat pheochromocytoma known to lack endogenous PC1. We found that the most likely site for the truncation event was at Arg⁹⁰⁰-Arg⁹⁰¹ since mutation of this site to Lys-His prevented processing of 87-kDa PC1. A PC1 mutant carboxy-terminally truncated at this site and expressed in PC12 cells was efficiently routed to the secretory pathway and stored in secretory granules, indicating that the carboxyl-terminal extension is not required for sorting of this enzyme. The function of the various PC1 constructs was assessed by analyzing pro-neurotensin cleavage to various forms. The carboxy-terminally truncated PC1 mutant was found to perform most of the cleavages of this precursor as well as wild-type PC1; however, the blockade mutant processed pro-neurotensin much less efficiently. Differences between the site preferences of the various enzymes were noted. Our results support the notion that carboxyl-terminal processing of PC1 serves to regulate PC1 activity.

We have shown previously that in AtT-20 cells, constitutively released PC1 is present mostly in the 87-kDa form, while PC1 (also known as PC3) released through stimulation predominantly consists of a carboxyl-terminally truncated, 66-kDa protein (Vindrola and Lindberg, 1992). This difference indicates that carboxy-terminal processing of 87-kDa PC1 probably largely occurs within regulated secretory vesicles. Recent studies employing temperature block, brefeldin A, and detergent of oligosaccharide maturation have supported the idea that PC1 is carboxy-terminally cleaved within the post-trans-Golgi network compartments in the regulated secretory pathway (Benjannet et al., 1992; Lindberg, 1994; Milgram and Mains, 1994; Zhou and Mains, 1994a). Through purification and characterization of recombinant PC1, we have demonstrated that both the 87- and the 74/66-kDa forms of PC1 are enzymatically active (Zhou and Lindberg, 1994). The conversion from 87-kDa PC1 to the 74/66-kDa forms not only increases specific activity while decreasing overall stability, it also narrows the pH optimum, increases calcium-dependence, and alters susceptibility to certain proteinase inhibitors (Zhou and Lindberg, 1994). Thus, we speculate that proteolytic processing of PC1 may play an important role in the regulation of PC1 enzymatic activity in vivo. The carboxy-terminal segment may therefore be involved in PC1 sorting into secretory vesicles. In the experiments described here, we have used site-directed mutagenesis to study the biosynthetic processing, sorting, and function of the various domains of PC1 in PC12 cells.

**Experimental Procedures**

Site-directed Mutagenesis of Mouse PC1—A mouse PC1/CMV expression vector was generously provided by Dr. N. G. Seidah (Benjannet et al., 1992). Site-directed mutations were designed to convert mPC1 Arg⁹⁰⁰-Arg⁹⁰¹ to Lys-His, Arg⁹⁰⁰-Arg⁹⁰¹ to Lys-Ala, or Gly⁵⁹² to a stop codon.

The mutation at Arg⁹⁰⁰-Arg⁹⁰¹ of mPC1 was carried out using the Mutagen™ in vitro mutagenesis kit (Bio-Rad). A mutagensizer primer, 5'-CTT TTC CAC TCC GTG CTT CTG ATT CTC GAC TG-3', was synthesized by LSU MRC Core Laboratories. Single-strand DNA was prepared as described by Ausubel et al. (1987). The mutation in mPC1591BL/CMV was confirmed by DNA sequencing using a DNA Sequenase™ version 2.0 kit (U. S. Biochemical Corp.). The mutations at Arg⁵⁹²-Arg⁵⁹³ and Gly⁵⁹² were performed using a PCR mutagenesis method. Primers XBAI-3 (5'-CAC AAC AAC TCT AGA CCC AGG AAC-3') and BSTXI-5 (5'-TAA ATG CCA AAG CTC TGG TGG-3') were synthesized to match the sequences of mPC1 cDNA at bp 1405-1426 or 2392-2415 (with a mutation switching CG to TA at 2404 bp to introduce an XbaI cleavage site). Two pairs of mutagenesis primers were synthesized to introduce mutations at Arg⁵⁹²-Arg⁵⁹³ or at Gly⁵⁹². These primers were 628BL-5 (5'-GGA AAA GAT GGT G-3') and 628BL-3 (5'-GGA AAA GAT GGT G-3'), which were digested with 1.5 µg of mPC1591BL DNA were transfected into JM101 Escherichia coli, then were amplified and purified using a Wizard™ Mini Prep DNA purification system (Promega, Madison, WI). About 2 µg of mPC1 cDNA and CMV DNA were digested with 1.5 µl of XbaI (30 units, New England Biolabs) in a 20-µl reaction mixture was further digested with 1.5 µl of EcoRI (15 units, New England Biolabs) in a 30-µl reaction mixture was further digested with 1.5 µl of EcoRI (15 units, New England Biolabs) in a 30-µl reaction mixture was further digested with 1.5 µl of EcoRI (15 units, New England Biolabs). This cDNA was used as the vector in the following ligation reaction. Using 0.5 µg of mPC1/CMV or mPC1591BL/CMV DNA as template, three separate PCR reactions were carried out. The

---

*This work was supported in part by National Institutes of Health Grant DA 05084 (to I. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: National Cancer Institute, National Institutes of Health, Bldg. 37, Rm. 4E16, Bethesda, MD 20892.

‡ Supported by a research scientist development award from NIDA. To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112. Tel.: 504-568-4799; Fax: 504-568-3370; E-mail: ilindb@lsumc.edu.

1 The abbreviations used are: CMV, cytomegalovirus; PCR, polymerase chain reaction; bp, base pair(s); DEAE, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; NT, neurotensin; i-1, immunoreactive.
Pulse-chase Experiments of PC12 Cells—
The ability of transfected mPC1s to process endogenous prohormone was assessed by measuring the production of mature and unprocessed neurotensin-derived peptides in PC12 cell extracts. PC12 cells were cultured and induced in 10-cm plates as described above. After 48 h of induction, the cells were washed three times with 10 ml of Dulbecco’s PBS, placed on ice, scraped into 1 ml of ice-cold 0.1 M HCl, and stored frozen at −70 °C. Upon thawing, the samples were vortexed to produce a homogeneous suspension. A 100-μl aliquot of cell extract was removed for protein determination and Western blotting using mPC1 antibodies (Fig. 2). The remainder was centrifuged on a microcentrifuge for 5 min, and the supernatant was removed and lyophilized. For the experiment shown in Fig. 5, the cell pellet obtained after removal of the HCl was solubilized in various amounts (between 0.3 and 0.6 ml) of Laemmli sample buffer containing 5 M urea to obtain a constant protein concentration of 3 mg/ml. Fifty μl of each sample were submitted to Western blotting using the monoclonal anti-terminal PC1 antibody and the blot subjected to video densitometry for quantitation of the amount of immunoactive PC1 in each plate. Proteins remaining in the gel following blotting were stained with Coomassie Blue; this procedure verified that the lanes had indeed been equally loaded with protein.

The anti-neurotensin antisera (Kislauskis et al., 1988) is depicted in Fig. 5a. It consists of a 169-residue polypeptide, which begins with a NH2-terminal signal peptide (1–22). Neurotensin is located near the COOH terminus of the precursor and is flanked by two Lys-Arg sequences at positions 148–149 and 163–164. Neurotensin is preceded by a neuropeptide sequence located between Lys433-Arg434 and Lys436-Arg437. Arg434 and Arg437 are the D and E protease cleavage sites, respectively, that are recognized by the E6I and K6L antibody. The latter peptide is produced by the K6L sequence is internal, whereas the former is generated by the iK6L antibody. The radioimmunoassay and reverse-phase HPLC procedures employed here to quantitate the various neurotensin-derived peptides have been fully described elsewhere (Bardit et al., 1993; Rovere et al., 1993).

The selection of the neurotensin, neuregulin N, E6I, and K6L antisera used here has been described previously in detail (Bardit et al., 1993; Rovere et al., 1993). Briefly, the neurotensin and E6I antisera react with the free COOH termini, while the neuregulin N and K6L antisera recognize the free NH2 termini, of their respective antigens. These antisera cross-react poorly (<1%) with antigenic sequences that are internal to proneuropeptide or proprotease fragments. Thus, the neurotensin and K6L antisera will detect all of the proprotease/terminal neurotensin sequence (including authentic neurotensin). Similarly, the E6I antisera will measure all of the precursor forms ending with the E6I sequence, while the neuregulin N and K6L antisera will assay the precursor products bearing NH2-terminal neuropeptide N and K6L sequences, respectively. The radioimmunoassay and reverse-phase HPLC procedures employed here to quantify the various neurotensin-derived peptides have been fully described elsewhere (Bardit et al., 1993; Rovere et al., 1993).

All cell extracts were directly assayed for their content in immunoreactive neurotensin (INT), E6I (E6I), and K6L (iK6L). Because of the above-described antisera specificity, the INT, E6I, and iK6L assays measure the amounts of precursor products that are processed at the Lys343-Arg344, Lys346-Arg347, and Lys348-Arg349 sequences, respectively. Portions of the cell extracts were submitted to Arg-directed tryptic digestion (Bardit et al., 1993; Rovere et al., 1993) and then assayed for immunoreactive K6L. The value of CTIK6L thus obtained provides an index of the total amount of proneuropeptide (either processed or unprocessed) that was synthesized and stored in the cells during the induction period. The remainder of the trypsin-treated samples was applied to reverse-phase HPLC, and the fractions were assayed for their immunoreactive neuropeptide N (iNN) content. Previous studies have shown that trypsin-generated INN can be resolved by HPLC into two peaks, one comigrating with synthetic neuropeptide N and the other with neuropeptide N bearing a COOH-terminal Lys-Arg extension (Bardit et al., 1993; Rovere et al., 1993). The latter peak is produced by Arg-directed tryptic digestion of precursor forms in which the neuropeptide N sequence is internal, whereas the former is generated by
cleavage of peptides that end with a COOH-terminal neuramidin N sequence. Thus, the post-HPLC assay of trypsin-generated neuramidin N provides a measurement of all the precursor products that are processed at the Lys$^{546}$-Arg$^{649}$ sequence (including authentic neuramidin N). The results were normalized for the amount of protein in each extract. The percentages of cleavage at the Lys$^{546}$-Arg$^{649}$, Lys$^{605}$-Arg$^{649}$, Lys$^{546}$-Arg$^{541}$, and Lys$^{546}$-Arg$^{652}$ sequences were calculated by dividing, respectively, INT, trypsin-generated neuramidin N, IEI, and IEI by CTIIK6L and by multiplying these ratio values by 100. Duplicate independent samples were analyzed on two separate occasions.

RESULTS

Site-directed Mutagenesis of PC1—It has been documented that four paired basic residues exist within the mPC1 carboxyl-terminal region, however, only Arg$^{590}$-Arg$^{591}$ and Arg$^{627}$-Arg$^{628}$ are conserved across human, mouse, rat, and angelfish PC1 (Seidah et al., 1991, 1992; Smeekens et al., 1991; Bloomquist et al., 1991; Roth et al., 1993). If 87-kDa PC1 is indeed cleaved at these two sites, the larger products should approximate 62 and 70 kDa, respectively; these molecular masses are close to the sizes of the PC1 forms obtained by spontaneous cleavage of 87-kDa PC1 (66 and 74 kDa; Zhou and Lindberg (1994)). Therefore, we theorized that these two sites could represent the cleavage sites for PC1 carboxyl-terminal processing. To test this hypothesis, site-directed mutagenesis reactions were carried out to convert Arg$^{590}$-Arg$^{591}$ to Lys-Ala or Arg$^{590}$-Arg$^{591}$ to Lys-His and Arg$^{627}$-Arg$^{628}$ to Lys-Ala, generating mPC1SB (single blockade at Arg$^{627}$-Arg$^{628}$) and mPC1DB (double blockade at both sites). A site-directed mutation to replace Gly$^{592}$ with a stop codon was also performed to generate mPC1ST, the carboxyl-terminally truncated form of PC1. These mutations are diagrammed in Fig. 1.

Biosynthesis of the Mutated PC1 Forms—In order to determine the effect of the mutations at the mPC1 carboxyl-terminal region, the carboxyl-terminal processing of wild-type mPC1 and the mutated forms (mPC1SB, mPC1DB, and mPC1ST) were studied in PC12 cells stably transfected with the various constructs. Western blotting using PC1 amino-terminal antiserum revealed that wild-type mPC1 and mPC1SB were largely converted to a 66-kDa form, while the majority of mPC1DB still remained as 87-kDa PC1 (Fig. 2), suggesting that mutation at Arg$^{590}$-Arg$^{591}$ (but not mutation at Arg$^{627}$-Arg$^{628}$) could effectively block PC1 carboxyl-terminal conversion. Western blotting also showed that mPC1ST (truncated at residues 592) exhibited a molecular mass identical to that of the 66-kDa PC1, and this form did not undergo any further carboxyl-terminal cleavage. Following stimulation of PC12 cells with 50 mM KCl, wild-type mPC1 as well as mPC1DB and mPC1ST could be released from regulated secretory pathway (Fig. 3). These data indicate that neither deletion of the carboxyl-terminal region (residues 592 to 726) nor mutations at Arg$^{590}$-Arg$^{591}$ and Arg$^{627}$-Arg$^{628}$ could block PC1 targeting into the regulated secretory pathway. It was noted that a small portion of mPC1DB was still cleaved, but that the cleavage product possessed a molecular mass slightly larger than that of cleaved wild-type mPC1, indicative of the involvement of an alternative cleavage site.

In pulse-chase labeling experiments, we found that amino-terminal conversions of pro-mPC1DB and pro-mPC1ST were completed within the first 20 min of synthesis, similar to wild-type pro-mPC1 (data not shown). This result indicates that substitution of Arg$^{590}$-Arg$^{591}$ and Arg$^{627}$-Arg$^{628}$ or deletion of the PC1 carboxyl-terminal region (residues 592-726) apparently had little effect on proPC1 conversion. During the later stages of biosynthesis, intracellular 87-kDa mPC1DB remained intact after a 4-h chase period, while wild-type PC1 was converted to the 66-kDa form (Fig. 4). Constitutive secretion of the 87-kDa form of both mPC1DB and wild-type PC1 occurred within 1 h after the pulse period (Fig. 4). Similar studies of the mPC1ST mutant demonstrated efficient synthesis of a 66-kDa form of PC1 and constitutive secretion into the medium over the same time period (results not shown).

Physiological Function of the Mutated PC1 Forms—PC12 cells are known to greatly increase their synthesis of pro-neurotensin under inducing conditions (Rovere et al., 1993). We have shown previously that transfection of mPC1 into PC12 cells can promote pro-neurotensin processing (Lindberg et al., 1994). To determine the physiological function of the mutated mPC1DB and mPC1ST, we compared the maturation of pro-neurotensin synthesized in PC12 cells transfected with the mutated mPC1DB and mPC1ST with that in PC12 cells expressing wild-type mPC1 and untransfected PC12 cells. It should be noted that the various cell lines expressed varying amounts of mPC1, with the double blockade mutant (mPC1DB) exhibiting the highest expression, and mPC1ST exhibiting the lowest. The relative amounts of PC1 in the cells used for this experiment were estimated by Western blotting a constant amount of protein from each dish used for neurotensin analysis and performing video densitometry of the blot. The ratios of the amount of mutant mPC1 (all forms) to wild-type mPC1 were

![Fig. 1. Diagram of site-directed mutants of mPC1.](http://example.com/fig1)

![Fig. 2. Western blotting of recombinant mPC1 proteins synthesized in PC12 cells.](http://example.com/fig2)

![Fig. 3. Western blotting of recombinant mPC1 proteins secreted from PC12 cells.](http://example.com/fig3)
approximately 0.3 (mPC1ST) and 9 (mPC1DB). As expected, untransfected PC12 cells exhibited no PC1 immunoreactivity.

The amount of proneurotensin (processed and unprocessed) stored in the various cell lines during the induction period ranged between approximately 10 and 30 pmol/mg protein (CTiK6L values are given in the legend to Fig. 5). Similarly to wild-type mPC1, mPC1DB and mPC1ST both possessed the ability to process proneurotensin (Fig. 5).

All forms of PC1 markedly increased proneurotensin cleavage at the Lys140-Arg141 and Lys148-Arg149 dibasic sites as compared to the control (Fig. 5b). They also cleaved, though less efficiently, the Lys384-Arg385 site. This cleavage event was not observed in control PC12 cells. Only mPC1 and mPC1ST were able to increase processing at the Lys383-Arg384 site above the level seen in the control, whereas mPC1DB appeared inactive in that respect. In general, and especially given the fact that it had the highest level of expression, mPC1DB was much less efficient in processing proneurotensin than mPC1 and mPC1ST. Interestingly, mPC1ST, the enzyme expressed at the lowest level, was the most active in processing the Lys383-Arg384 site, in contrast to mPC1DB which apparently did not cleave this dibasic despite its high level of expression. Thus, there appear to be certain differences in proneurotensin processing efficiency and site usage between the 66- and 87-kDa PC1 forms.

**DISCUSSION**

PC1 is known to be cleaved within its carboxyl-terminal region at a late stage of its biosynthesis (Vindrola and Lindberg, 1992). Previous work has suggested that an autocatalytic mechanism may be involved in this process (Zhou and Lindberg, 1994); however, the site of this carboxyl-terminal cleavage event has not yet been identified. In this work, we have assumed that this cleavage occurs at a paired basic site, since these are known to represent consensus sequences for PC1 cleavage. Four paired basic residues are located in the mPC1 carboxyl-terminal region; cleavage at these sites can generate products with estimated molecular masses between 62 and 73 kDa. However, among these sites, only Arg590-Arg591 and Arg592-Arg593 are conserved among the PC1 sequences of humans, rat, mouse, and anglerfish (Seidah et al., 1991, 1992; Smeekens et al., 1991; Bloomquist et al., 1991; Roth et al., 1993); thus, these two sites were thought to represent likely candidate sites for PC1 carboxyl-terminal cleavage. By performing site-directed mutagenesis at these two sites, we found that mutation of Arg592-Arg593 alone had little effect on the generation of the 66-kDa form, while mutations of both Arg590, Arg591 and Arg592-Arg593 were able to block the conversion of 87-kDa PC1 to the 66-kDa form. Furthermore, mPC1ST (truncation at Gly592) exhibited a molecular mass on SDS-PAGE identical to that of endogenous 66-kDa PC1 converted from the 87-kDa wild-type mPC1. Subsequent to the generation of our mutants, the sequence of Aplysia PC1 was published (Chun et al., 1994); PC1a from this species contains the first of these dibasics, but not the second. Taken together, these data strongly suggest that Arg590-Arg591 is the major cleavage site for the generation of 66-kDa PC1 in vivo. Since wild-type PC12 cells possess a regulated secretory pathway, but lack the ability to process prohormones at paired basic residues, PC1 cleavage at Arg590-Arg591 within PC12 cells is likely to be attributable to an autocatalytic mechanism. This interpretation is supported by our in vitro work (Zhou and Lindberg, 1994) and in vivo results obtained in AtT-20 cells, which indicate that overexpression of PC1 results in increased COOH-terminal proteolytic processing (Zhou and Mains, 1994a).

The presence of an intermediate form of PC1 of approximately 74 kDa has been observed in in vitro studies (Zhou and Lindberg, 1994); however, little 74-kDa mPC1 is found in PC12 cells (this study) or in AtT-20 cells (Vindrola and Lindberg, 1992; Milgram and Mains, 1994). Taken together with the finding of lesser effects of the mutation of Arg627-Arg628 in PC12 cells, these results suggest that 74-kDa PC1 may represent a minor product during the carboxyl-terminal processing of PC1 in vivo. A possible explanation for these differences is that the cleavage site generating the 74-kDa form is blocked in vivo, possibly due to an association of PC1 carboxyl-terminal region with membrane or with other proteins. The association of PC1 with membranes and association with other granule proteins have both been reported (Vindrola and Lindberg, 1992; Palmer and Christie, 1992).

Although mutation at Arg590-Arg591 and Arg627-Arg628 substantially blocked PC1 carboxyl-terminal conversion, a small portion of 87-kDa mPC1DB was still cleaved. The product was slightly larger than wild-type 66-kDa PC1 on SDS-PAGE, sug-
suggesting that an alternative cleavage site is involved. Through limited digestion using chymotrypsin, trypsin, and subtilisin, which possess different substrate specificities, we found that all three proteinases were able to convert 87-kDa recombinant PC1 to 66- and 74-kDa-like products in its carboxy-terminal region (Zhou and Lindberg, 1994). These results suggest that the cleavage site in the PC1 carboxy terminus is located in an exposed region which can readily be attacked. Therefore, the alternative cleavage site usage in PC12 cells may be due to the action of other proteinases located in the regulated secretory pathway. Alternatively, PC1 itself may also act at alternative cleavage sites. This idea is supported by our in vitro studies that demonstrate spontaneous carboxy-terminal cleavage of 87-kDa mPC1DB purified from Chinese hamster ovary cells amplified for the production of this protein (results not shown).

A possible alternative cleavage site for transfected mPC1 may be Lys602-Arg603, although this site is present only in mouse PC1. Cleavage at the Lys602,Arg603 site can generate a product 12 amino acids longer than the product cleaved at Arg590,Arg591 (66-kDa PC1); this molecular mass is also consistent with our observed molecular masses on SDS-PAGE. Construction of a PC1 vector encoding a further mutation at Lys602 will be required to investigate this possibility.

Carboxy-terminal conversion of PC1 occurs mainly in regulated secretory granules, as evidenced by previous studies in AtT-20 and PC12 cells (Vindrola and Lindberg, 1992; Benjannet et al., 1992; Lindberg et al., 1994; Lindberg, 1994; Milgram and Mains, 1994; Zhou and Mains, 1994a). However, the functional significance of this conversion event is not clear. The activation of proPC1 occurs within the endoplasmic reticulum (Lindberg, 1994; Milgram and Mains, 1994; Goodman and Gorman, 1994), while peptide hormone precursors are thought to be cleaved within the later stages of the secretory pathway (reviewed by Loh et al. (1992)). It may thus be necessary for the cell to regulate PC1 function during intracellular transport. The decreasing pH gradient from the endoplasmic reticulum to the secretory granules may represent one important aspect of this regulation. The pH within the trans-Golgi network and regulated secretory granules correlates well with the optimal pH of PC1 activity, between 5.0 and 6.5 (Zhou and Lindberg, 1993; Jcan et al., 1993; Rufaut et al., 1993). On the other hand, since the timing and location of PC1 carboxy-terminal processing coincide with the timing and location of prohormone processing, truncation of PC1 may also play a role in the regulation of enzyme activity. In vitro studies have shown that carboxy-terminal cleavage of PC1 dramatically increases PC1 activity against peptide and prohormone substrates (Zhou and Lindberg, 1994), suggesting that carboxy-terminal cleavage of PC1 could potentially possess physiological significance.

In order to determine the function of the PC1 carboxy-terminal region in vivo, we compared the processing of proneurotensin in PC12 cells stably transfected with wild-type mPC1, mutated mPC1DB, or mPC1ST. In contrast to AtT-20 cells, PC12 cells do not express prohormone convertases; thus, neurotensin is stored mainly in precursor form (Carraway et al., 1993; Rovere et al., 1993). When the varying expression levels are taken into account, the 66-kDa form of PC1 was found to be the most active against proneurotensin, especially relative to the 87-kDa blockade mutant, which was expressed at much higher levels. The comparatively low activity of mPC1DB against proneurotensin confirms our previous in vitro results, which indicate that the 87-kDa PC1 represents only a partially active PC1 form (Zhou and Lindberg, 1994); removal of the carboxy-terminal region appears to be required to fully activate PC1. The finding that the various forms of PC1 are differentially active against proneurotensin supports our in vitro results showing that the 87- and 74/66-kDa recombinant PC1s exhibit differing specific activities (Zhou and Lindberg, 1994).

A recent study has also demonstrated that expression of carboxy-terminally truncated PC1 (Stop5619) in AtT-20 cells increases the rate of conversion of proopiomelanocortin (Zhou and Mains, 1994b). Based on these in vivo and in vitro observations, we speculate that carboxy-terminal processing of PC1 during transport through the secretory pathway may control the amount of PC1 activity available for the processing of prohormones.

In conclusion, we have demonstrated that PC1 carboxy-terminal conversion largely occurs at Arg590,Arg591 site through a possible autocatalytic mechanism; the PC1 carboxy-terminal domain (Gly392 to Asn726) is required neither for activation nor for intracellular transport of PC1 to secretory granules. However, removal of this domain appears to increase total PC1 activity and alters cleavage site preference. In line with our previous in vitro data, these results support the idea that carboxy-terminal processing is important for the regulation of PC1 function.

Acknowledgments—We are grateful to L. Efferink and R. Scheller for supplying PC12 cells and N. G. Seidah for Rpc1 M4 encoding mPC1. We thank J. F. Finley for expert assistance with cell culture and C. BiU for capable assistance with Western blots.

REFERENCES

Aubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Smith, J., A., Seidman, J. G., and Struhl, K. (eds) (1987) Current Protocols in Molecular Biology, John Wiley & Sons, New York.

Benjannet, S., Reudelhuber, T., Mercure, C., Rondeau, N., Chretien, M., and Seidah, N. G. (1992). J. Biol. Chem. 267, 11417–11423.

Bidard, J., de Nadail, F., Rovers, C., Mainier, D., Lau, J., Martinez, J., Cuber, J., and Kitabgi, P. (1993) Biochem. J. 291, 225–233.

Bloomquist, B. T., Eipper, B. A., and Mains, R. E. (1991) Mol. Endocrinol. 5, 2014–2024.

Carraway, R. E., Bullock, B. P., and Dobner, P. R. (1993) Peptides 14, 991–999.

Chun, J. Y., Kerner, J., Kreiner, T., Scheller, R. H., and Axel, R. (1994) Neuron 12, 833–844.

Goodman, L. J. and Gorman, C. M. (1994) Biochem. Biophys. Res. Commun. 201, 795–804.

Jeen, F., Basak, A., Rondeau, N., Benjannet, S., Hendy, G. N., Seidah, N. G., Chretien, M., and Laure, C. (1993) Biochem. J. 292, 891–900.

Kidaukis, E., Bullock, B., McNeil, S., and Dobner, P. R. (1988) J. Biol. Chem. 263, 4963–4968.

Laemmli, U. K. (1970) Nature 227, 680–685.

Lindberg, I. (1994) Mol. Cell. Neurosci. 5, 263–268.

Lindberg, I. and Zhou, Y. (1995) Methods Neurosci. 23, 94–108.

Lindberg, I., Ahn, S., and Breslin, M. B. (1994) Mol. Cell. Neurosci. 5, 614–622.

Loh, Y. P. (1992) in Mechanisms of Intracellular Trafficking and Processing of Propeptides (Loh, Y. P., ed) pp. 181–183, CRC Press, Boca Raton, FL.

Milgram, S., and Mains, R. E. (1994) J. Cell Sci. 107, 737–745.

Palmr, D. J. and Christie, D. L. (1992) J. Biol. Chem. 267, 19806–19812.

Roth, W. W., Mackin, R. B., and Nae, D. B. (1993) Endocrinology 11, 331–140.

Rovers, C., Nadail, F. D., Bidard, J., Cuber, J., and Kitabgi, P. (1993) Peptides 14, 862–868.

Rufaut, N., Brennan, S. O., Hawks, D. J., Dixson, J. E., and Birch, N. P. (1993) J. Biol. Chem. 268, 20291–20298.

Seidah, N. G., Marconkievicz, M., Benjannet, S., Gaspar, L., Basubeg, G., Mattei, M. G., Laure, C., Mbakay, M., and Chretien, M. (1991) Mol. Endocrinol. 5, 111–122.

Seidah, N. G., Hamelin, J. W., Day, R., and Chretien, M. (1992) DNA Cell Biol. 11, 263–269.

Smeekens, S. P., Avruch, A. S., LaMendola, J., Chan, S. J., and Steiner, D. F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 340–344.

Vindrola, O., and Lindberg, I. (1992) Mol. Endocrinol. 6, 1088–1094.

Zhou, Y., and Lindberg, I. (1993) J. Biol. Chem. 268, 5615–5623.

Zhou, Y., and Lindberg, I. (1994) J. Biol. Chem. 269, 18408–18413.

Zhou, A., and Mains, R. E. (1994a) J. Biol. Chem. 269, 17440–17447.

Zhou, A., and Mains, R. E. (1994b) Soc. Neurosci. Abstr. 20, 447.
Mutational Analysis of PC1 (SPC3) in PC12 Cells: 66-kDa PC1 IS FULLY FUNCTIONAL
Yi Zhou, Carole Rovere, Patrick Kitabgi and Iris Lindberg

J. Biol. Chem. 1995, 270:24702-24706.
doi: 10.1074/jbc.270.42.24702

Access the most updated version of this article at http://www.jbc.org/content/270/42/24702

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 25 references, 11 of which can be accessed free at http://www.jbc.org/content/270/42/24702.full.html#ref-list-1