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Biochemistry

PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues

(prohormone convertases/β-lipotropin/corticotropin/β-endorphin/α-melanotropin)

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ABSTRACT **A recombinant vaccinia virus vector was used to coexpress the two candidate mouse prohormone convertases, PC1 and PC2, together with mouse proopiomelanocortin (POMC) in the constitutively secreting cell line BSC-40 and in the endocrine tissue-derived cell lines PC12 and AtT-20, which exhibit regulated secretion. Monitoring of POMC processing demonstrated the distinct cleavage specificities of PC1 and PC2, since in the cell lines analyzed (*i*) PC1 cleaves POMC into corticotropin and β-lipotropin, (*ii*) PC2 cleaves POMC into β-endorphin, an N-terminally extended corticotropin containing the joining peptide, and either αMSH or desacetyl-αMSH, and (*iii*) PC2 cleaves POMC at the five pairs of basic residues analyzed, whereas PC1 cleaves two of them preferentially, suggesting that PC2 has a broader spectrum of activity than PC1. These data are consistent with our hypothesis on the physiological role of PC1 and PC2 as distinct proprotein convertases acting alone or together to produce a set of tissue-specific maturation products in the brain and in peripheral tissues.**

Limited proteolysis of inactive precursors at pairs of basic residues or, less frequently, at single basic amino acids (1, 2) is a general mechanism by which the cell produces a variety of active proteins and peptides. Until recently, the molecular nature of the processing enzyme(s) was not identified except for the yeast pro-α mating factor precursor, where a subtilisin-like serine proteinase called KEX2 was proven to be the physiological convertase (3). In the last year, different groups obtained the complete cDNA structures of three distinct candidate mammalian convertases, furin (4), PC1 (5, 6), and PC2 (5, 7). The putative convertase function of these proteins was largely based on sequence homology with KEX2 (8, 9) and on their tissue and cellular distribution, especially for PC1 and PC2 (5, 6). Here we show that the expression of PC1 or PC2 by vaccinia virus (VV) recombinants results in the production of distinct proteolytic enzymes in three cell lines: the mouse pituitary corticotroph line AtT-20 and two that do not express these enzymes endogenously, the African green monkey kidney epithelial cell line BSC-40 and the rat pheochromocytoma cell line PC12. These proteinases cleave proopiomelanocortin (POMC) into the same set of distinct peptides known to be produced *in vivo* in the hypothalamus, adenohypophysis (AP), and pars intermedia of the pituitary, including adrenocorticotropin (ACTH), β-lipotropin (βLPH), β-endorphin (βEnd), and α-melanotropin (αMSH) or desacetyl-αMSH.

MATERIALS AND METHODS

VVs. Purified recombinant VVs using the full-length mouse (m) PC1 and mPC2 cDNA inserts (5, 6) (VV:mPC1 and VV:mPC2) were prepared as described (10). VV:POMC (mouse) was a gift of G. Thomas (Vollum Institute, Portland, OR).

Northern Blot Analysis. Total RNA was prepared as described (11). The [α-^{32}P]UTP-labeled cRNA probes were generated from cDNA sequences of mPC1 (6) and mPC2 (5) inserted in plasmid Rc/CMV vector (Invitrogen, San Diego) and linearized with *Kpn* I to yield an mPC1 probe of 520 bases (specific activity, 97,500 Ci/mmol; 1 Ci = 37 GBq) and an mPC2 probe of 1750 bases (specific activity, 247,000 Ci/mmol), both containing the 3′ end of the cDNAs.

VV Infections. Cells were infected with a mixture of VV:POMC and either VV:WT (wild-type control), VV:mPC1, VV:mPC2, or both VV:mPC1 and VV:mPC2 at various multiplicities of infection, since VV infection results in a significant decrease (up to 91%) in the production of endogenous POMC (12). After the infection period, the inoculum was replaced with either Dulbecco's modified Eagle's medium (DMEM) or Eagle's minimum essential medium (MEM) and the cells were incubated for 17 hr at 37°C. The cells were then incubated in DMEM or MEM containing 0.5% fetal bovine serum for 8 hr and subsequently extracted in 5 M acetic acid containing iodoacetamide (0.3 mg/ml) and phenylmethylsulfonyl fluoride (0.3 mg/ml).

Biosynthesis. Following VV infection and 17 hr of incubation, cells from 25-cm^2 dishes were incubated at 37°C for 8 hr in either methionine-, leucine-, phenylalanine-, or tryptophan-free RPMI-1640 (GIBCO) medium containing 500 μCi of L-[^{35}S]methionine, L-[^{3}H]leucine, or L-[^{3}H]phenylalanine or 25 μCi of L-[^{14}C]tryptophan (DuPont). For immunoprecipitations, we used AT-1 and AT-2, polyclonal antibodies prepared in this laboratory that recognize ACTH- or βEnd-containing peptides, respectively. The final pellet was resuspended in Laemmli sample buffer with 100 mM dithiothreitol. The boiled samples were then electrophoresed in 15% polyacrylamide gel containing 0.1% SDS. Microsequencing of radiolabeled peptides was performed with an Applied Biosystems sequenator (13).

Peptide Analysis. The media and cellular extracts were chromatographed on a Brownlee Lab Aquapore RP-300 C_8 reverse-phase (RP) HPLC column (0.7 × 25 cm) equilibrated with 0.1% trifuoroacetic acid in 20% acetonitrile. Proteins were eluted with a linear gradient of 20–60% acetonitrile in 80 min at 2 ml/min. The dried collected fractions (2 ml) were subsequently analyzed by RIAs for βEnd, ACTH, and αMSH. The ACTH RIA was performed using antibody AT-1, which recognizes POMC and ACTH-(1–17). Similarly, the βEnd RIA was performed using AT-2, which recognizes POMC, βLPH, and βEnd. An αMSH-specific RIA was performed using an antibody recognizing the C-terminally amidated αMSH and desacetyl-αMSH (a gift of H. Akil and

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Abbreviations: POMC, proopiomelanocortin; ACTH, adrenocorticotropin; βLPH, β-lipotropin; βEnd, β-endorphin; αMSH, α-melanotropin; JP, joining peptide; AP, anterior pituitary; NIL, neurointermediate lobe; VV, vaccinia virus; ir, immunoreactive; RP, reverse-phase; pfu, plaque-forming units.

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S. Watson, University of Michigan). POMC-related products purified by RP-HPLC were analyzed by SDS/15% PAGE, as described (14).

RESULTS

Northern Blot Analysis. BSC-40 cells infected with VV:mPC1 and VV:mPC2 at 5 plaque-forming units (pfu) per cell expressed PC1 (Fig. 1*A*, lane 3) and PC2 (Fig. 1*B*, lane 3) transcripts of appropriate size to have initiated at the 7.5 K promoter and terminated at the VV thymidine kinase termination site (15). Of the three cell lines used, only AtT-20 cells (Fig. 1, lanes 4) expressed PC1 and PC2 mRNAs [≈20:1 ratio, as already observed (5)]. AtT-20 PC1 mRNA is of equal size to NIL and AP PC1 mRNA, while AtT-20 PC2 mRNA is slightly (≈100 bases) larger than its pituitary counterpart. After correction for the specific activity of each probe used, quantitative scanning of the Northern blots allowed us to estimate that in both mouse and rat pituitary, the PC1/PC2 transcript ratio within the diverse cells of the AP is about 3–5:1, whereas in the NIL it is about 1:15, emphasizing the observed (5, 6) reverse order of enrichment of PC1 and PC2 mRNAs within the AP and NIL, respectively.

POMC Processing by PC1 or PC2 in BSC-40 Cells. We assessed the processing capability of PC1 and PC2 by coinfecting BSC-40 cells with VV:POMC and either VV:mPC1, VV:mPC2, or VV:WT (control). The media and cell extracts were purified on RP-HPLC and the chromatographic fractions were analyzed for βEnd and ACTH immunoreactivity (Fig. 2). Consistent with the constitutive nature of the BSC-40 cells (11), little intracellular storage of POMC products was observed in the cells coinfected with either VV:mPC1 or VV:mPC2 (Fig. 2 *B* and *D*). The POMC-derived peptides were observed in the media of cells infected with VV:mPC1 or VV:mPC2 (Fig. 2 *A* and *C*). PC1 produced a major ir βEnd product that was eluted at 37% and a minor one at 35.5% acetonitrile, whereas PC2 did the opposite (Fig. 2*A*). PC1 also produced major ir ACTH peptides eluted at 30% and 32.5% acetonitrile and a minor peptide at 37%. PC2 produced major ir ACTH products eluted at 37% acetonitrile, with minor ones eluted at 30% and 32.5% (Fig. 2*C*).

The ir peptides purified by RP-HPLC (Fig. 2 *A* and *C*) were individually analyzed by SDS/PAGE (Fig. 3). Thus, the material under the ir βEnd peak produced by PC1 (Fig. 2*A*) migrated with an apparent molecular mass of 13 kDa (Fig. 3*A*). In contrast, PC2 processed POMC into a major peptide (eluted at 35.5% acetonitrile, Fig. 2*A*) comigrating with synthetic βEnd-(1–31) peptide, and a minor one migrating at 13 kDa, similar to the one produced by PC1 (Fig. 3*D*). The major ir ACTH peptides produced by PC1 and eluted at 30% and 32.5% acetonitrile (Fig. 2*C*) migrated on SDS/PAGE at 15 and 14 kDa, respectively (Fig. 3*B*). The major ir ACTH peptides produced by PC2 and eluted at 37% acetonitrile (Fig. 2*C*) migrated at 17.5 kDa (Fig. 3*E*). Finally, the products that were eluted at 40% acetonitrile (Fig. 2) contained either unprocessed POMC, as in the case of the control coinfection of VV:POMC with VV:WT (Fig. 3*F*) or processing intermediates migrating at 31 and 27 kDa (Fig. 3*C*). The 37- and 39-kDa forms represent POMC glycosylated at one or both N-glycosylation sites (16, 17). In agreement with an earlier observation (16) that not all glycosylated variants of POMC are processed equally, we note that both glycosylated forms were preferentially processed by PC1 (Fig. 3*C*) and PC2 (data not shown) compared with the nonglycosylated 35-kDa form (Fig. 3 *C* and *F*).

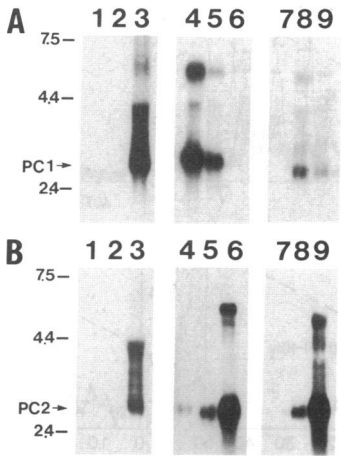

FIG. 1. Northern blot analysis of total RNA (10 μg per lane, unless otherwise stated). (*A*) mPC1 cRNA probe. Lanes: 1, BSC-40; 2, BSC-40/VV:WT; 3, BSC-40/VV:mPC1; 4, AtT-20; 5, mouse AP (5 μg); 6, mouse neurointermediate lobe (NIL, 1 μg); 7, PC12; 8, rat AP; 9, rat NIL (5 μg). (*B*) mPC2 cRNA probe. Lanes: 1, BSC-40; 2, BSC-40/VV:WT; 3, BSC-40/VV:mPC2; 4, AtT-20; 5, mouse AP (5 μg); 6, mouse NIL (1 μg); 7, PC12; 8, rat AP; 9, rat NIL (2.5 μg). Exposure time was 18 hr for each lane except for *B* lanes 1–3, where exposure was for 1 hr. Markers are in kilobases.

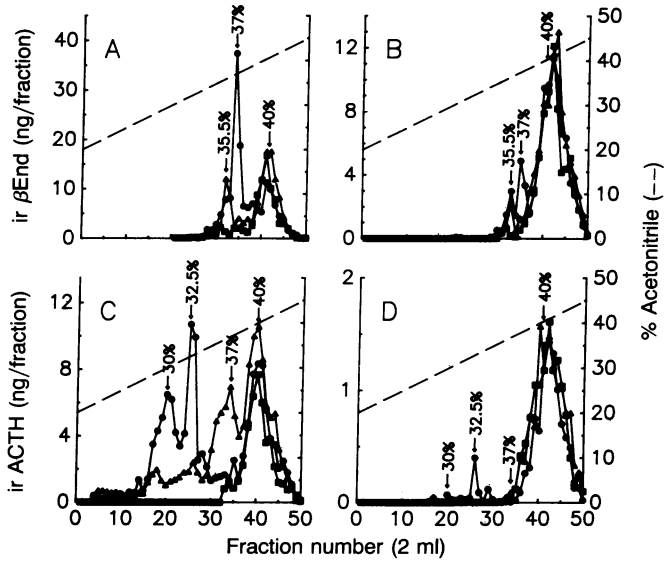

FIG. 2. POMC processing by PC1 or PC2 in BSC-40 cells. BSC-40 cells (2.7×10^7) grown in 145-cm^2 plates were coinfected with VV:POMC at 1 pfu per cell and with either VV:mPC1 (●), VV:mPC2 (▲), or VV:WT (■) at 2 pfu per cell. Medium (*A* and *C*) and cell (*B* and *D*) extracts were chromatographed on a C_8 RP-HPLC column, and aliquots of the fractions were analyzed by either βEnd (*A* and *B*) or ACTH (*C* and *D*) RIA.

PC1 and PC2 Cleavage Sites of POMC. Fig. 4 depicts the autoradiogram obtained after SDS/PAGE of the radiolabeled βEnd and ACTH immunoprecipitates of the media of coinfected BSC-40 and PC12 cells. In BSC-40 and PC12 cells, PC1 cleaved POMC into a 13-kDa ir βEnd peptide (lanes 1 and 9), whereas PC2 generated a 4-kDa peptide (lanes 2 and 10). The sequence of the 13-kDa peptide revealed leucine at positions 2, 8, 10, and 14 and the absence of methionine and phenylalanine in the first 20 residues, confirming that PC1 produces βLPH by cleavage of POMC at Glu-Phe-Lys-Arg^{138}↓ (18). The Phe^4-Met^5 sequence of the 4-kDa peptide (lanes 2 and 10) proves that this product is βEnd obtained by the cleavage of POMC at Lys-Asp-Lys-Arg^{178}↓ (18).

The sequence of radiolabeled peptides produced by PC1 and immunoprecipitated by the ACTH antibody revealed the presence of Met^4 and Phe^7, which, together with the results of Figs. 3*B* and 4 (lane 6), confirms that ACTH-(1–39) is produced by this enzyme by the cleavage of POMC at both Glu-Gly-Lys-Arg^{97}↓ and Glu-Phe-Lys-Arg^{138}↓ sequences.

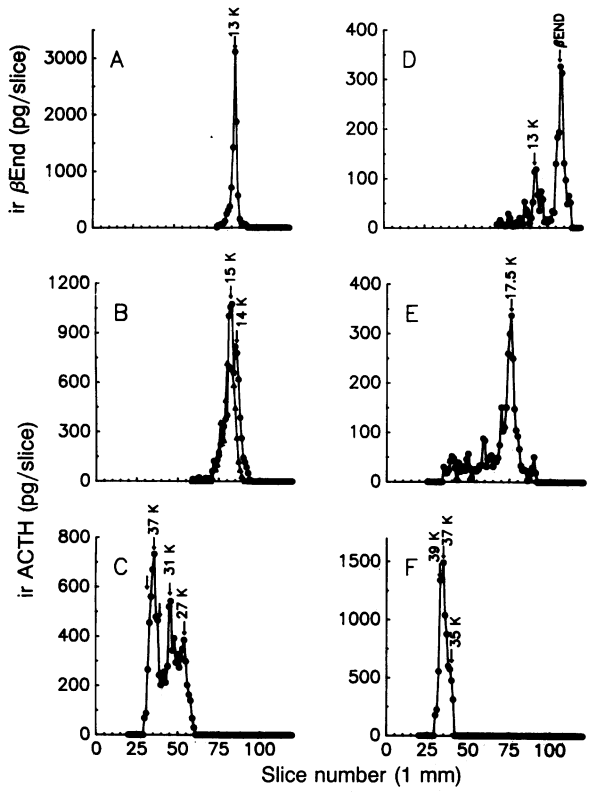

FIG. 3. SDS/PAGE of POMC products produced by PC1 or PC2 in BSC-40 cells. The βEnd (*A* and *D*) and ACTH (*B*, *C*, *E*, and *F*) RIA results are normalized to picograms (pg) of immunoreactivity per gel slice. The chosen peptide products were immunoreactive (ir) βEnd peptides (Fig. 2*A*) produced by VV:mPC1 and eluted at 37% acetonitrile (*A*) or VV:mPC2 and eluted at 35.5% acetonitrile (*D*); ir ACTH peptides (Fig. 2*C*) produced by VV:mPC1 and eluted at 30% (▲) or 32.5% (●) acetonitrile (*B*), by VV:mPC1 and eluted at 40% (*C*), by VV:mPC2 and eluted at 37% (*E*), or by VV:WT (control) and eluted at 40% (*F*). Unlabeled arrows in *C* point to the migration positions of the 39- and 35-kDa POMC forms.

Figs. 2*C* and 3*E* show that the major ir ACTH peptide produced by PC2 is different from ACTH, as it is eluted at 37% acetonitrile and migrates at 17.5 kDa, which is 2.5–3.5 kDa greater than ACTH (mixture of 14 and 15 kDa, Fig. 3*B*). Further, the absence of detectable N-terminal ACTH sequence in the [^{35}S]methionine and [^{3}H]phenylalanine-labeled peptides immunoprecipitated by the ACTH antibody suggests that this ACTH-containing peptide is an N-terminally extended form of ACTH. The finding of Trp^{7} in the sequence of the equivalent peptide obtained from PC12 cells confirms that this peptide contains the 19-amino acid joining peptide (JP) and the Lys-Arg^{97} pair connecting this peptide to ACTH (18). These results demonstrate that PC2 cleaves POMC at the Ala-Gln-Arg-Arg^{76}↓ and Glu-Phe-Lys-Arg^{138}↓ sequences (18) to produce the JP-ACTH product.

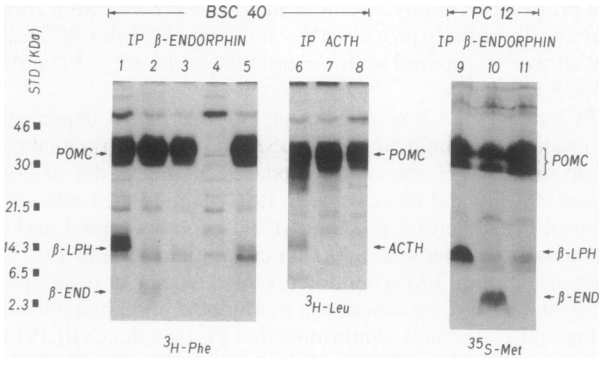

FIG. 4. SDS/PAGE of biosynthetic products. The immunoprecipitated (IP) products obtained following coinfection of VV:POMC at 1 pfu/cell with either VV:mPC1 (lanes 1 and 6), VV:mPC2 (lanes 2 and 7), or VV:WT (lanes 3 and 8) at 2 pfu per cell. Lanes 4 and 5, immunoprecipitates obtained from control infections of BSC-40 cells with VV:WT (3 pfu per cell) and VV:POMC (2 pfu per cell), respectively. Lanes 9–11, βEnd-containing peptides obtained from PC12 cells and produced by the coinfection of VV:POMC at 2 pfu/cell with either VV:mPC1 (lane 9), VV:mPC2 (lane 10), or VV:WT (lane 11) at 4 pfu per cell.

Processing of POMC by PC1 and PC2 in PC12 Cells. Northern blot analysis of PC12 cells demonstrated the absence of detectable PC1 and PC2 transcripts (Fig. 1). When PC12 cells were coinfected with VV:POMC and VV:mPC1 we observed a much higher level of βLPH (peak at 37% acetonitrile) than in control cells coinfected with VV:WT, both intracellularly (Fig. 5*B*) and in the medium (Fig. 5*A*). Similarly, cells coinfected with VV:mPC2 produced much more βEnd (35.5% acetonitrile; Fig. 5 *A* and *B*) compared with control cells. With respect to ACTH, PC1 produced mostly ACTH-(1–39) (30%, 32.5%, and 34% acetonitrile; Fig. 5 *C* and *D*). JP-ACTH (37% acetonitrile) was the major ir ACTH product obtained by PC2 (18 kDa on SDS/PAGE and Trp^{7} in sequence). Interestingly, in PC12 cells the intracellular forms of ACTH were eluted at 30%, 32.5%, and 34% acetonitrile (12 kDa on SDS/PAGE; data not shown), whereas the extracellular forms were eluted at 30% and 32.5% (14 and 15 kDa). Therefore, both glycosylated, phosphorylated ACTH and nonglycosylated ACTH are stored in PC12 cells (Fig. 5*D*), whereas only glycosylated, phosphorylated ACTH is released from this cell line under nonstimulating conditions. As opposed to the medium (Fig. 5 *A* and

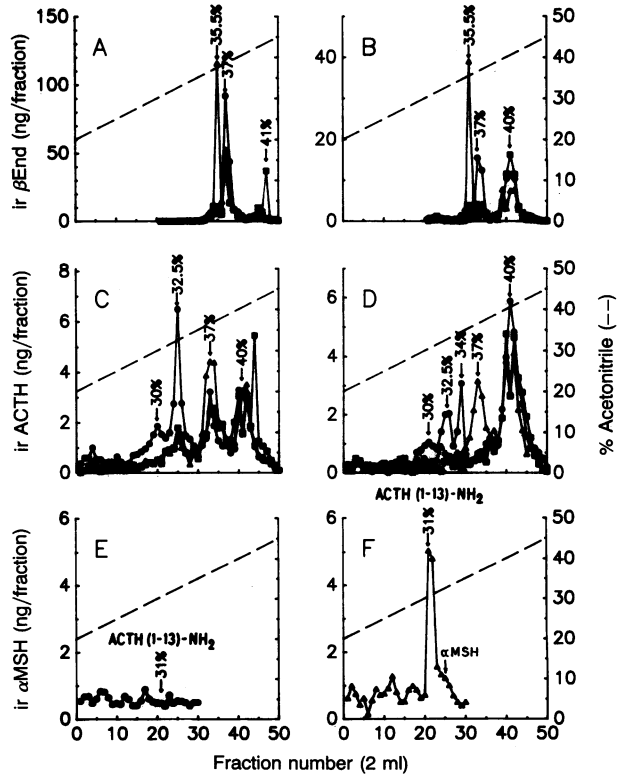

FIG. 5. POMC processing by PC1 and PC2 in PC12 cells. PC12 cells (3.5×10^{7}) grown in 60-cm^{2} plates were coinfected with VV:POMC at 2 pfu per cell and either VV:mPC1 (●), VV:mPC2 (▲), or VV:WT (■) at 4 pfu per cell. Medium (*A* and *C*) and cell (*B*, *D–F*) extracts were chromatographed and analyzed by βEnd (*A* and *B*), ACTH (*C* and *D*), or αMSH (*E* for PC1 and *F* for PC2) RIA. Elution positions of αMSH (33% acetonitrile) and desacetyl-αMSH [ACTH-(1–13)-NH$_2$] (31%) are shown.

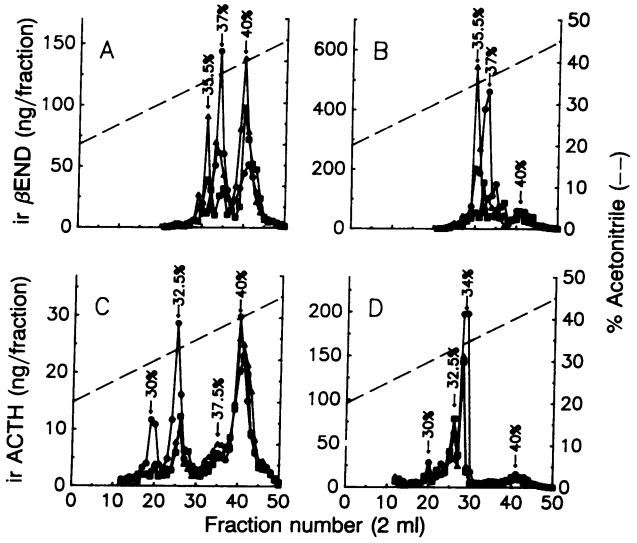

FIG. 6. POMC processing by PC1 or PC2 in AtT-20 cells. AtT-20 cells (3.6×10^7) grown in 145-cm^2 plates were coinfected with VV:POMC at 2 pfu per cell and either VV:mPC1 (●), VV:mPC2 (▲), or VV:WT (■) at 4 pfu per cell. Medium (*A* and *C*) and cell (*B* and *D*) extracts were chromatographed and analyzed by βEnd (*A* and *B*) or ACTH (*C* and *D*) RIA.

C), little intracellular processing was observed in control cells (Fig. 5 *B* and *D*). This suggests that no significant storage of products processed by the endogenous convertase(s) occurs in PC12 cells.

Finally, unlike PC1 (Fig. 5*E*) and control (data not shown), PC2 generated desacetyl-αMSH from POMC (Fig. 5*F*). This result shows that PC12 cells cannot N-acetylate the ACTH-(1–13)-NH_2 produced by PC2, probably reflecting the absence of the required N-acetyltransferase. Further, as compared to the control infection with only PC1, when PC12 cells infected with VV:POMC were coinfected with both VV:PC1 and VV:PC2, the production of desacetyl-αMSH was associated with a concomitant decrease in ACTH-(1–39), and βLPH was almost completely transformed into βEnd (data not shown).

Processing of POMC by PC1 and PC2 in AtT-20 Cells. AtT-20 cells showed PC1 and PC2 mRNAs at an estimated 20:1 ratio (Fig. 1, lanes 4). Above this endogenous activity, coinfection of VV:POMC with either VV:mPC1 or VV:mPC2 enhanced the processing capability, to produce higher levels of the same products as those found in the control infection with VV:WT. As seen from Fig. 6 *A* and *B*, within both the medium and the cells, the increase in βEnd-containing cleavage products was seen mostly in the βLPH product (37% acetonitrile; Figs. 2*A* and 3*A*). In contrast to PC1, the increase in βEnd-containing cleavage products by PC2 was represented mostly by βEnd, which was eluted at 35.5% acetonitrile (Figs. 2*A* and 3*D*). Therefore, in AtT-20 cells, both enzymes enhance the cleavage of POMC by the endogenous convertases to yield the same βEnd-containing products as those obtained in BSC-40 and PC12 cells.

The intracellular form of ACTH (Fig. 6*D*) was eluted at 34% acetonitrile, whereas the extracellular forms were eluted at 30% and 32.5%. As expected from the ACTH-(1–39) structure, the [^{35}S]methionine sequence of all three forms showed Met^4. Furthermore, SDS/PAGE gave 15, 14 and 12 kDa for the forms that were eluted at 30%, 32.5%, and 34% acetonitrile (data not shown). In agreement with an earlier report (19), our results show that the nonglycosylated ACTH (12 kDa) is the major storage form of the peptide in AtT-20 cells, whereas the N-glycosylated and phosphorylated forms (14 and 15 kDa) are preferentially secreted by these cells under nonstimulated conditions. Lastly, in contrast to PC1, we notice no enhancement of ACTH-containing products by PC2 over control (Fig. 6 *C* and *D*). This is due to the almost complete PC2 processing of ACTH into αMSH (eluted at 33% acetonitrile; data not shown). Therefore, unlike PC12 cells (Fig. 5*F*), AtT-20 cells contain the required N-acetyltransferase to produce αMSH.

DISCUSSION

Fig. 7 shows that PC1 and PC2 (5, 6) are separate proteinases, selectively cleaving POMC at distinct pairs of basic residues. Within POMC, PC2 is capable of cleaving on the C-terminal side of all the five pairs of basic residues analyzed—namely Ala-Gln-Arg-Arg^{76}↓, Glu-Gly-Lys-Arg^{97}↓, Gly-Lys-Lys-Arg^{114}↓, Glu-Phe-Lys-Arg^{138}↓, and Lys-Asp-Lys-Arg^{178}↓. PC1 preferentially cleaves POMC at Glu-Gly-Lys-Arg^{97}↓ and Glu-Phe-Lys-Arg^{138}↓, with minor cleavage at Lys-Asp-Lys-Arg^{178}↓ to produce βEnd. However, we cannot rule out the possibility of a cleavage by PC1 at the Ala-Gln-Arg-Arg^{76}↓ sequence, since we did not analyze for the JP fragment (20). PC1 principally generates ACTH and βLPH, whereas the efficient production of αMSH and βEnd requires the action of PC2. Both proteinases can produce substrates for the amidation enzyme (21), since they can cleave C-terminal to either Gly-Lys-Arg↓ or Gly-Lys-Lys-Arg↓.

Our data indicate a physiological role for PC1 in the processing of POMC in the AP. In support of this hypothesis, PC1 generates ACTH and βLPH as the major final POMC products (Fig. 7), both of which are known to represent the most abundant POMC-derived peptides in the AP. Even though some βEnd was detected in the AP, it is interesting

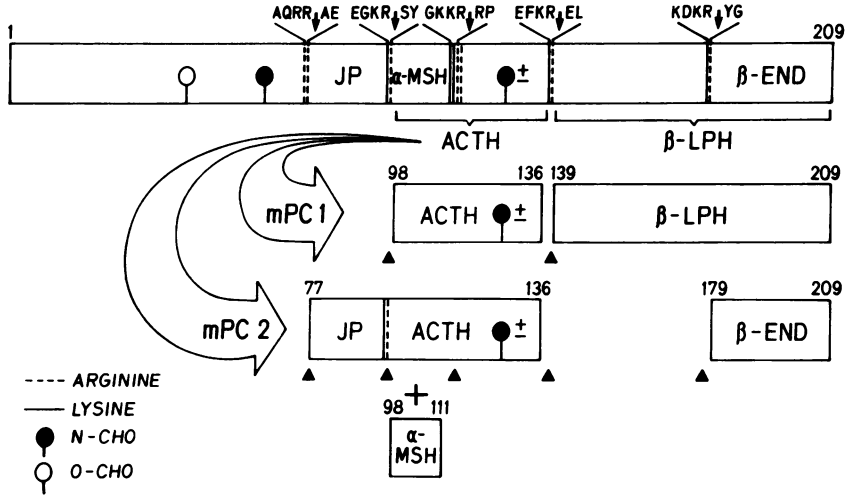

FIG. 7. Major end products of POMC processing by PC1 and PC2. Arrows represent the cleavage sites following pairs of basic residues (single-letter amino acid symbols). Black triangles tally the cleavage sites of each enzyme. Numbers represent the start and end positions of the processed peptides based on the reported POMC sequence (18). The N- and O-glycosylation (CHO) sites as well as the pairs of basic residues are emphasized.

that no significant production of αMSH was found (22). Therefore, it is possible that in the AP, PC1 is the convertase responsible for the minor βEnd production. The comparative cellular localization of PC1 and PC2 in the AP corticotrophs should help in the verification of this hypothesis. In the pars intermedia, the major POMC products are αMSH and βEnd-derived peptides (13, 16, 23). In agreement we find that PC2 mRNA is much more abundant than PC1 mRNA in the NIL (ref. 6 and Fig. 1). Therefore, our data are consistent with actions of both PC1 and PC2 in this tissue to efficiently process POMC into its final physiological products.

Our work raises questions concerning the physiological participation of PC1 and PC2 in the processing of various proprotein precursors. The tissue distribution of the mRNAs coding for PC1 and PC2 suggests that their synthesis is restricted to endocrine and neuroendocrine cells (5, 6). In the brain, PC2 has a much wider distribution pattern than PC1 (6), which together with its observed broader cleavage specificity profile suggests a more extended physiological role in the processing of many neuropeptides. The data presented in this work on POMC processing should be extended to other proprotein precursors in order to define in more detail the cleavage specificity of these proteinases, and whether they are also able to cleave proproteins at monobasic sites. Preliminary data on plasma prekallikrein activation in AtT-20 cells demonstrated that neither PC1 nor PC2 can activate plasma prekallikrein [requiring cleavage C-terminal to the monobasic Ala-Arg↓ bond (14)].

Although we did not identify the intracellular localization of PC1 and PC2, the processing observed within the three cell lines used (Figs. 2, 5, and 6) implies that the conversion of POMC occurs intracellularly. Both PC1 and PC2 are proteinases capable of cleaving POMC in the absence of storage granules, as observed in the constitutively secreting BSC-40 cells (Fig. 2). Further, in cells with regulated secretion such as PC12 and AtT-20, our results demonstrated that PC1 and PC2 process POMC to the same products as in BSC-40 cells. It has been proposed that within endocrine cells capable of storing active peptides, prohormones transit in a region of the cell in which processing can occur, but that within the environment of the mature storage granules, no further endoproteolysis occurs (24).

Furin represents a membrane-bound, third member of the subtilisin-like processing enzyme candidates (4). Overexpression of furin has been claimed to cleave POMC into βLPH in BSC-40 cells (25) and to activate pro-von Willebrand factor in COS-1 cells (26). Although control PC12 cells do not express PC1 or PC2 (Fig. 1), they are able to process POMC into βLPH (Fig. 5*A*) and ACTH (Fig. 5*C*) but do not store these processed POMC products (Fig. 5 *B* and *D*). This suggests that in PC12 cells processing is performed within the constitutive route. Recent data show that COS-1 (26) and BSC-40 and PC12 (27) cells contain furin. The latter may be involved in the conversion of POMC in PC12 cells, and possibly prosomatostatin in COS-1 cells (28). It is likely that together PC1 and PC2, perhaps in combination with other convertases such as furin, can fashion the final maturation products of proproteins in a tissue-specific manner (5, 6).

Recent yeast KEX2 expression studies suggest that KEX2 is first synthesized as a preproprotein that undergoes N-terminal proteolytic removal of the prosegment within the endoplasmic reticulum (R. Fuller, personal communication). The activation site seems to be one of the two Lys-Arg pairs found within the first 85 amino acids of the pro-KEX2 structure. A similar sequence occurs twice within the N-terminal 80–84 amino acids of furin (4), PC1 (6), or PC2 (5). The cleavage of POMC by PC1 and PC2 presented here suggests either that translation of PC1 or PC2 mRNA results in an active enzyme directly or that zymogen activation takes place early during intracellular transport. Our preliminary data on the expression of PC1 and PC2 in the baculovirus system show that both enzymes are synthesized as active proteinases in insect Sf9 cells. Determination of the N-terminal sequence of the active enzymes should specify the site of zymogen activation.

Although we have demonstrated the cleavage selectivity of PC1 and PC2 for pairs of basic residues in POMC, further characterization of their cleavage specificity spectrum is needed. It would be interesting to see whether members other than PC1, PC2, and furin can be found within this growing family of subtilisin-like proteinases in eukaryotes.

Note Added in Proof. Since the submission of this article, the full sequence of an AtT-20 cell-derived cDNA called PC3 has been reported (29). This sequence is virtually identical to that of mPC1 (6).

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