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Comparative Tissue Distribution of the Processing Enzymes "Prohormone Thiol Protease," and Prohormone Convertases 1 and 2, in Human PTHrP-Producing Cell Lines and Mammalian Neuroendocrine Tissues

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Peptide hormones are generated by proteolytic processing of their respective protein precursors by several prohormone processing proteases. The peptide hormone PTHrP is widely expressed in normal and malignant tissues, where proPTHrP undergoes proteolytic processing to generate PTHrP peptides with distinct biological actions. In this study, the tissue distribution of the prohormone processing enzymes PTP, PC1, and PC2 were compared by immunohistochemistry in human PTHrP-producing cancer cell lines, and in mammalian neuroendocrine and other tissues from rat and bovine that contain peptide hormones. PTP, PC1, and PC2 were prominently expressed in PTHrP-expressing human cancer cell lines originating from tumors of the breast, lung, prostate, as well as lymphoma. These processing enzymes also showed significant expression in normal mammalian neuroendocrine tissues from bovine and rat, including pituitary, hypothalamus, adrenal medulla, pancreas, and other tissues. Most neuroendocrine tissues contained prominent levels of at least two of the three processing enzymes examined, and all tissues contained at least one of these three enzymes. Differential expression of processing enzyme proteins was also demonstrated by Western blots. The differential expression of PTP, PC1, and PC2 observed in certain cancer and normal neuroendocrine cell types postulates selective roles for these processing enzymes in different tissues for generating biologically active peptide hormones. These results support the importance of these processing enzymes in their hypothesized roles in prohormone processing.

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Introduction

Parathyroid hormone-related protein (PTHrP) and numerous peptide hormones, as well as peptide neurotransmitters, are first synthesized as prohormone precursors that undergo proteolysis to generate the smaller, biologically active peptide forms *(1–3)*. Prohormone processing is known to occur within the regulated secretory pathway of neuroendocrine cells. The prohormone undergoes cellular trafficking through the rough endoplasmic reticulum (RER) and Golgi apparatus, and is then packaged into immature secretory vesicles. The majority of proteolytic steps in prohormone processing occur within secretory vesicles during their maturation, with some processing in the RER and Golgi apparatus. The mature secretory vesicles contain fully processed prohormone in the form of biologically active peptide hormones that are secreted to regulate specific physiological functions. Proteolytic processing of prohormones, at paired basic or monobasic residues, generates diverse peptide hormones with distinct physiological actions.

PTHrP (parathyroid hormone-related protein) is a polypeptide that undergoes such proteolytic processing. Overproduction of PTHrP occurs in common human cancers including those of the lung, breast, and prostate *(4)*. These cancer cells produce high levels of heterogeneous PTHrP-related peptide products with distinct effects on serum calcium, cell growth, and skeletal cell function *(5)*. Both bone and cartilage cells produce and respond to neuroendocrine factors such as PTHrP *(6)*.

Proteases of distinct active site classes have been demonstrated to participate in prohormone processing. The largest identified group of prohormone convertases (PC) is represented by the family of subtilisin-like proteases that possess homology to yeast kex2 and bacterial subtilisin *(2,3,7)*.

In particular, PC1 and PC2 represent neuroendocrine-specific members of the prohormone convertases that have been demonstrated to be involved in processing POMC, proinsulin, and other prohormones. More recently, the cysteine protease known as 'prohormone thiol protease' (PTP) has been shown to be involved in processing proenkephalin within secretory vesicles of adrenal medullary chromaffin cells *(8–10)*. PTP has been characterized as a unique protease complex consisting of three cysteine protease activities. In addition, an aspartyl protease known as 'POMC converting enzyme' (PCE) has been demonstrated within pituitary secretory vesicles for the conversion of POMC into ACTH, α-MSH, and β-endorphin *(11–13)*. These findings implicate multiple proteases of different active site classes in the production of structurally and functionally diverse peptide hormones.

Comparisons of the relative tissue distribution of these different processing proteases is a first step in studying the parallel or differential roles of such proteases in the production of peptide hormones in pathological and normal neuroendocrine cell types. Notably, the cysteine protease PTP demonstrates high specific activities with prohormone and peptide–MCA substrates *(14–16)*, cleaves recombinant proenkephalin and peptide substrates at appropriate paired basic residue sites *(8,9)*, and is localized to regulated secretory vesicles of neuroendocrine chromaffin cells *(17)*. Therefore, in this study, the tissue distribution of the novel cysteine protease PTP was compared with the subtilisin-like prohormone convertases 1 and 2 in human PTHrP-producing cancer cell lines and neuroendocrine tissues that produce peptide hormones. Our results demonstrated similar and differential tissue distributions of PTP, PC1, and PC2, which support a role for these proteases in prohormone processing.

Methods and Materials

Immunocytochemistry of PTP, PC1, and PC2 in Cell Lines and Tissues

Immunocytochemical localization of the processing enzymes PTP, PC1, and PC2 was evaluated in neuroendocrine cell lines and tissues, including skeletal tissue, that express PTHrP and other peptide hormones. These enzymes were examined in PTHrP-expressing human cancer cell lines originating from lung cancer (A549, BEN, NCI-H441, NCI-H478, NCI-H727, NCI-H1385 cell lines), B-cell lymphomas (ML107, RAJI, RPMI1788, HBL-1, HBL-3 cell lines), breast cancer (BT-20, MDA-MB415, MDA-MB435S, MDA-MB453, MCF-7, T-47D cell lines), prostate cancer (PNT1A, PPC-1, PC-3 lines), medullary thyroid cancer (TT), and neuroblastoma (SK-N-BE2) *(18,19)*. These cell lines were cultured as previously described *(20)*. In addition, processing enzymes were examined in human cartilage tissues from normal and osteoarthritic conditions *(6)*. The processing enzymes PTP, PC1, and PC2 were also examined in normal bovine and rat neuroendocrine tissues that included adrenal, brain, hypothalamus, intestine, kidney, lung, pancreas, parathyroid, pituitary, spleen, and thymus.

For immunocytochemistry, cells and tissues were formalin-fixed and parraffin-embedded as described previously *(21)*. Rat and bovine tissues were collected from freshly sacrificed animals, washed in PBS and placed into Bouin's fixative for >24 hours. Tissues were dehydrated, equilibrated in xylene and embedded into paraffin, and sections were cut with a microtome (5 μm thick). Sections were placed onto ProbeOn Plus microscope slides (Fisher Scientific, Pittsburgh, PA), baked at 65ºC overnight, deparaffinized, treated with Endo/Blocker (Biomedia Corp., Foster City, CA), and hydrated through a series of isopropyl alcohol solutions (100%, 95%, 70%, and 35%) with equilibration in distilled water for 5 min. Slides were incubated in 1% zinc sulfate and microwaved for two 5 min bursts, blocked with a nonspecific protein solution (20% FBS, 0.25% gelatin, 0.01% azide in PBS), and sections were incubated with protein-A purified antibodies (10–20 μg IgG/mL at 4ºC) that detect PTP, PC1, and PC2 *(17,22)*. Biotinylated goat anti-rabbit IgG antibody was applied to sections for 1 h, followed by streptavidin-alkaline phosphatase enzyme complex for 1 h at rt. Slides were developed in 0.38 *M* bromochloroindolyl phosphate/0.4 *M* nitroblue tetrazolium in Tris-buffered saline, pH 9.5, for 5–15 min, and then dehydrated through a series of isopropyl alcohols (35%, 70%, 95%, and 100%) with equilibration in xylene. For controls, PTP, PC1, or PC2 antibodies were preincubated overnight with the peptide antigens used to generate these antibodies (10 μg peptide/mL) *(17,22)* before immunostaining of tissue sections. Immunostaining was observed with an AO standard light microscope using 40X and 100X objectives, and images were recorded with a Diagnostic Instruments SPOT digital camera system (Sterling Heights, Michigan).

Relative Scoring of Immunohistochemical Cellular Staining of PTP, PC1, and PC2 Processing Enzymes

Positive immunostaining was assessed semi-quantitatively by scoring the proportion of cells showing positive immunoreactivity *(23)* with (+) = 1–33% cells showing positive immunostaining; (++) = 34–66% positive cells; (+++) = 67–100% positive cells. At least five representative fields were evaluated.

Analysis of Processing Enzyme Proteins by Western Blots

Western blots of tissue samples (10 μg aliquots) were performed by SDS-PAGE of the tissue sample, electrophoretic transfer to nitrocellulose membranes, and immunoblotting with anti-PTP (1:500 final dilution), anti-PC1 (1:500), and anti-PC2 (1:500) serum for the three enzymes, as described previously *(17,22)*.

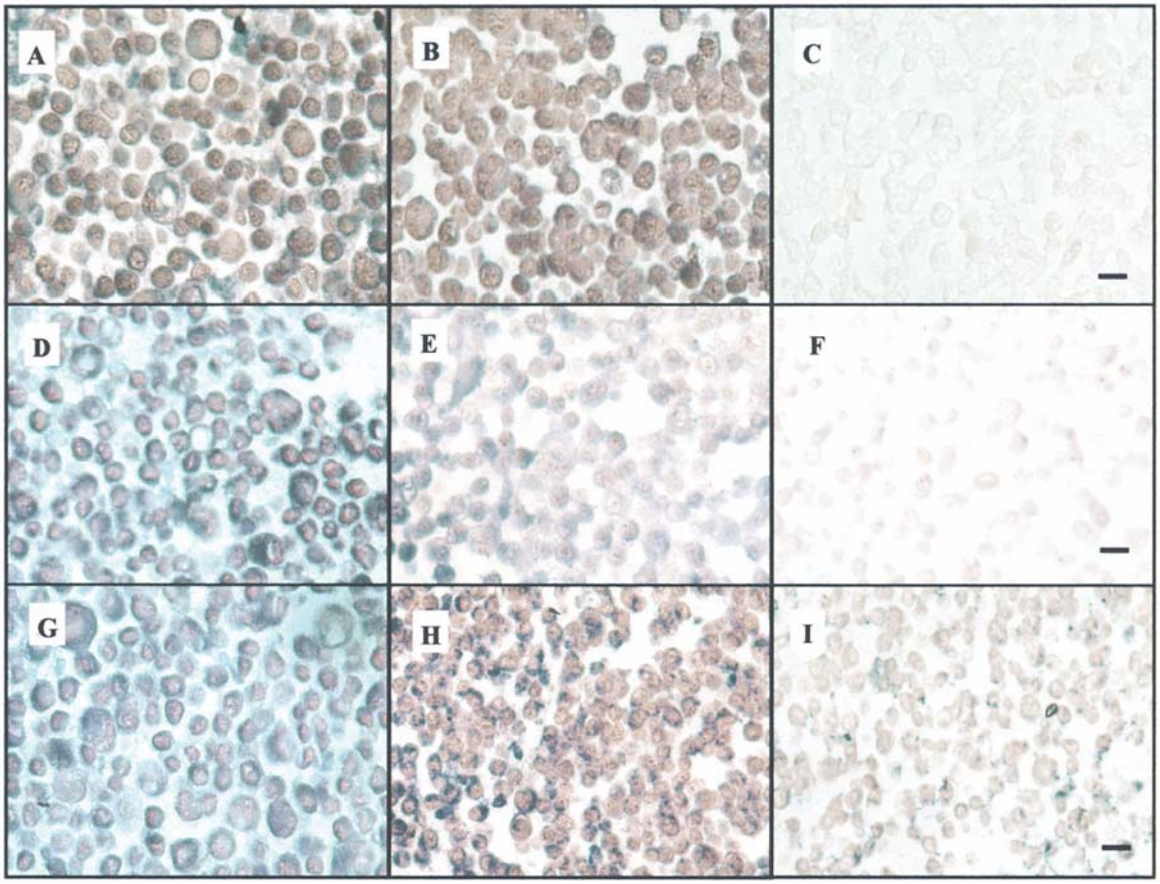

Fig. 1. PTP, PC1, and PC2 processing enzyme immunoreactivities in human PTHrP-producing human cancer cell lines. *PTP immunostaining.* PTP immunohistochemistry was performed with MDA-MB453 and MDA-MD435 human breast cancer cell lines in panels A and B, respectively. The far right panel (C) shows the absence of PTP immunostaining when the IgG antibody against PTP was preincubated with peptide antigen *(17)*, demonstrating specificity of the PTP immunostaining. *PC1 immunostaining.* PC1 immunohistochemistry was conducted with MDA-MB453 and A549 cell lines originating from human breast and lung tumors, respectively. MDA-MB453 and A549 cells with PC1 immunostaining are shown in panels D and E, respectively. The far right panel (F) shows the antigen absorption control, with reduction of PC1 immunostaining with preincubation of the PC1 antibody with the peptide antigen used to generate the PC1 antiserum *(22)*. *PC2 immunostaining.* PC2 immunohistochemistry was performed on MDA-MB453 and SK-N-BE-2 cell lines originating from human breast tumor and neuroblastoma, respectively. Panels G and H show immunostaining in MDA-MB453 and SK-N-BE-2 cells, respectively. The far right panel (I) shows the immunoabsorption control, with preincubation of the PC2 antibody with PC2 peptide antigen *(22)* prior to incubation with tissue sections. The distance bar indicates 50 μm.

Results

Comparison of PTP, PC1, and PC2 Neuroendocrine Distribution in Human PTHrP-Producing Cancer Cell Lines and Tissues

To assess the presence of the prohormone processing enzymes PTP, PC1, and PC2 in PTHrP-containing cell lines and neuroendocrine tissues, as well as skeletal tissue, the presence of these cysteine and subtilisin-like proteases in such cell lines and tissues was evaluated by immunohistochemistry. PTP immunostaining in human breast cancer cell lines, MDA-MB453 and MDA-MB435, was demonstrated (Fig. 1A,B). PTP immunostaining was present in cytoplasmic regions of the cells; this pattern of cellular staining is consistent with previous results demonstrating PTP localization in cytoplasmic secretory vesicles *(17)*. Absorption of PTP immunostaining with peptide antigen (Fig. 1C) demonstrated specificity of immunostaining. These immunohistochemical images in the breast cancer cell lines were representative of PTP immunostaining in human cancer cell lines derived from tumors of the lung, breast, prostate, and lymphomas (B-cell) (Table 1).

The relative scoring of the proportion of cells containing enzyme immunoreactivities in various cell lines is shown in Table 1, with scores of (+), (++), and (+++) indicating the percent of positive cells from 1–33%, 34–66%, and 67–100% of total cells, respectively. PTP was prominently detected in the majority of the lung cancer cell lines. All of the breast cancer cell lines (6 lines), as well as the prostate cancer cell lines (3 cell lines), were positive for PTP. Differential

Table 1
Differential Distribution of PTP, PC1, and PC2 in Human PTHrP-Producing Cell Lines and Tissues

Cell Type	Cell Line	PTP	PC1	PC2
Lung Cancer	A549	+++	+++	–
“	BEN	+++	+++	++
“	NCI-H441	+++	+++	–
“	NCI-H478	+++	+++	+
“	NCI-H727	+++	+++	++
“	NCI-H1385	++	++	+
Breast Cancer	BT-20	+++	+++	–
“	MDA-MB415	+++	+++	+++
“	MDA-MB435S	+++	+++	–
“	MDA-MB453	+++	+++	+
“	MCF-7	+++	+++	+
“	T-47D	+++	+++	+++
Prostate Cancer	PPC-1	+++	+++	-
“	PC-3	++	++	+++
Prostate Epithelial (transformed)	PNT1A	+++	++	++
B-Cell Lymphoma	ML107	NT	–	+++
“	RAJI	NT	–	+++
“	RPMI1788	+++	+	–
“	HBL-1	+	+	–
“	HBL-3	–	+++	+
Neuroblastoma	SK-N-BE2	+++	++	++
Medullary Thyroid Cancer	TT	+++	+++	+
Cartilage, normal		++	+++	++
Cartilage, osteoarthritic		+++	+++	++

Relative enzyme immunoreactivities were scored as (+) = 1-33% cells showing positive immunostaining; (++) = 34-66% positive cells; (+++) = 67-100% positive cells, as described in the methods. NT, not detectable, and essentially not detected was indicated as (–).

expression of PTP was observed in several B-cell lymphoma cell lines. RPMI1788 cells were strongly positive for PTP, and the other lymphoma cell lines contained little or no detectable PTP. PTP was also detected in a human neuroblastoma cell line. Moreover, cartilage and chondrocyte tissues that produce PTHrP also contained PTP immunoreactivity. Overall, PTP shows positive immunoreactivity in numerous human PTHrP-producing cancer cell lines and several tissues.

Comparison of PC1 and PC2 enzyme immunoreactivities in these PTHrP-producing cell lines and tissues indicated both similarities and differences in distribution compared to PTP. PC1 immunoreactivity was observed in the cytoplasmic region of breast and lung cancer cell lines MDA-MB453 and A549, respectively, with lower staining in apparent nuclear areas (Fig. 1D,E). Similarly, PC2 immunostaining was observed in MDA-MB453 and SK-N-BE2 cells (Fig. 1G,H), PTHrP-containing human cell lines that originate from breast and neuroblastoma, respectively. These cellular patterns of PC1 and PC2 were representative of those for other human cancer cell lines and tissues examined. Relative abundance of different cell lines showing PC1 or PC2 enzyme immunostaining was assessed by semi-quantitative scoring (Table 1).

The majority of the lung cancer cell lines were strongly positive for PC1, with somewhat lower abundance of PC1 in the NCI-H1385 line. PC2 in these lung cancer cells, however, showed lower relative abundance compared to PC1; several cell lines (A549, NCI-H441, and NCI-H727) showed little PC2. The breast cancer cell lines, like the lung cancer cells, also showed abundant PC1 immunostaining, with variations in PC2 immunostaining that ranged from very positive in MDA-MB415 and T-47D cells, to low or moderate immunostaining in MDA-MB453 and MCF-7 cell lines, and little immunostaining in BT-20 and MDA-MD435S cells. Two prostate cell lines showed abundant PC1 immunostaining, and lower levels of PC2 immunoreactivity. Interestingly, several B-cell lymphoma cell lines showed either high levels of PC1 with lower levels of PC2 (HBL-3), or high PC2 immunoreactivity with low levels of PC1 (ML107 AND RAJI). Cartilage tissue contained both PC1 and PC2. Overall, these results demonstrate similarities and differences in the relative distribution of PTP with PC1 and PC2 in human PTHrP-producing cell lines and tissues.

Comparison of PTP, PC1, and PC2 Tissue Distribution in Rat and Bovine Neuroendocrine Tissues

In rat, PTP immunohistochemistry was demonstrated in the anterior lobe of the pituitary (Fig. 2A), as well as in intermediate and posterior pituitary lobes (Table 2). In the anterior lobe, PTP immunostaining was primarily observed in cytoplasmic areas (Fig. 2A). PC1 and PC2 were also found in the anterior lobe (Table 2). In intermediate pituitary, abundant PTP and PC2 were observed, with lower PC1 immunostaining. The posterior lobe contained both PTP and PC1, with less abundant PC2. Further studies showed PTP immunoreactivity in rat brain, which also contained PC1 and PC2. PTP was present in both type I and II rat epithelial cells, but only type II lung tissue contained one of the PC enzymes, PC1. In pancreatic islet cells, PC1 and PC2 were present, with lower PTP immunostaining; pancreatic acinar cells showed somewhat lower levels of these enzymes. It is of interest that rat GH3 cells, a rat cell line derived from pituitary, demonstrated prominent PTP immunoreactivity, but little PC1 or PC2. Thus, some differences in relative abundance of PTP, PC1, and PC2 were observed. However, the majority of results demonstrate co-localization of PTP with PC1 and PC2 processing enzymes in neuroendocrine cells from rat tissues.

Bovine tissues were abundant in PTP, PC1, and PC2 immunoreactivities and demonstrated differential tissue distributions of these processing enzymes. PTP immunostaining was present in bovine adrenal medulla (Fig. 2B), which

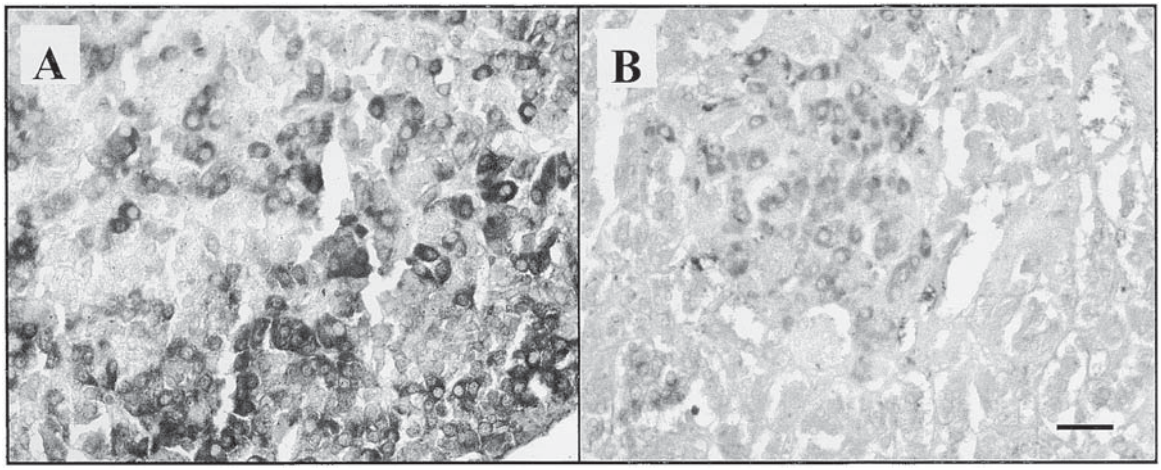

Fig. 2. PTP immunohistochemistry in rat pituitary and bovine adrenal medulla. *A. PTP immunostaining in rat pituitary.* PTP immunoreactivity was observed in tissue sections of rat anterior pituitary. *B. PTP immunostaining in bovine adrenal medulla.* PTP immunoreactivity was demonstrated in tissue sections of bovine adrenal medulla. The distance bar indicates 75 μm.

Table 2
Comparative Neuroendocrine Tissue
Distribution of PTP, PC1, and PC2 in Selected Rat Tissues

Tissue	Cell Type	PTP	PC1	PC2
Pituitary	Anterior	+++	++	+++
"	Intermediate	+++	–	+++
"	Posterior	++	++	–
"	GH3	+++	NT	NT
"	AtT-20	++	+/–	+
Adrenal	Medullary	++	–	++
"	Cortical	++	++	+
Brain	Neural elements	+++	++	+
Lung	Type I	+++	–	–
"	Type II	+++	+++	–
Pancreas	Islets	+	+++	++
"	Acinar	+	–	+/–
Kidney	numerous	+++	+++	+++

Relative enzyme immunoreactivities were scored as (+) = 1-33% cells showing positive immunostaining; (++) = 34-66% positive cells; (+++) = 67-100% positive cells, as described in the methods. NT, not detectable, and essentially not detected was indicated by (–).

Table 3
Neuroendocrine Tissue Distribution
of PTP, PC1, and PC2 in Selected Bovine Tissues

Tissue	Cell Type	PTP	PC1	PC2
Adrenal	Medullary	++	+	+
"	Cortical	+++	++	+++
Hypothalamus	Neural elements	+++	+++	+++
Pituitary	Anterior	+	+++	++
"	Intermediate	+	++	+++
"	Posterior	++	+++	+++
Pancreas	Islets	–	+++	+
"	Acinar	++	–	+
Spleen	Lymphocytes	++	++	++
Thymus	Hassal's corpuscles (squamous reticular cells)	–	+++	–
	Lymphocytes	+++	–	+
Parathyroid	Principal	++	–	+
Thyroid	Follicular	–	+++	+++
Intestine	Goblet	–	++	–
Kidney	Glomeruli	++	–	–

Relative enzyme immunoreactivities were scored as (+) = 1-33% cells showing positive immunostaining; (++) = 34-66% positive cells; (+++) = 67-100% positive cells, as described in the methods. Immunoreactivity that was essentially not detected was indicated by (–).

is known to contain PTP activity *(8)*. Adrenal medulla contained lower levels of PC1 and PC2 immunoreactivities (Table 3), which is consistent with the presence of PC1 and PC2 activities in bovine adrenal medulla at lower levels compared to PTP *(9,22,24)*. In addition, the adrenal cortex was positive for PTP, PC1, and PC2 immunoreactivity. The hypothalamus was especially abundant in all three processing enzymes, which is consistent with the multitude of peptide hormones and neurotransmtiters synthesized in this brain region. In pituitary, the posterior lobe contained moderate PTP immunostaining, and PTP was also detected in anterior and intermediate lobes. PC1 and PC2 were quite abundant in all three lobes of the pituitary. It is of interest that pancreatic islet cells did not contain detectable PTP, but PC1 and PC2 were present, which are known to be involved in converting proinsulin to insulin *(3)*. Pancreatic acinar cells, on the other hand, contain PTP and PC2, with lower abundance of PC1. Splenic lymphocytes contain all three processing enzymes, which may participate in producing POMC-derived peptides *(25)*. Notably, thymic lymphocytes displayed prominent PTP, and lower PC1 or PC2. These results demonstrate that multiple neuro-

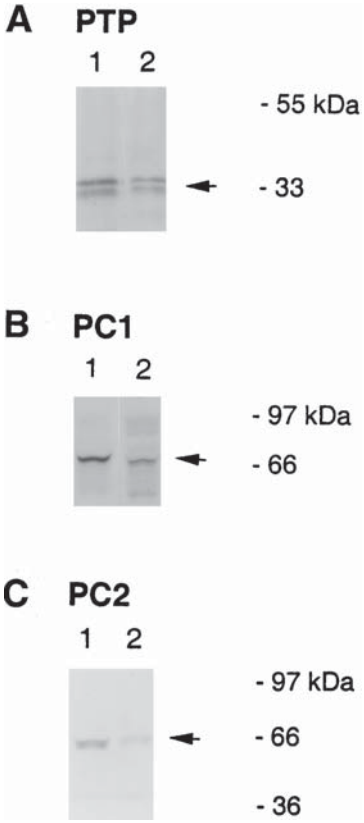

Fig. 3. Relative levels of processing enzymes in Western blot analyses. Relative levels of processing enzymes were assessed by Western blots for PTP, PC1, and PC2 in panels A, B, and C, respectively. In panel A, PTP immunoblots of rat pituitary and adrenal are shown as a 33 kDa band (arrow) in lanes 1 and 2, respectively. In panel B, PC1 in human MDA-MB415 and PC-3 human cancer cell lines is shown as a band of 66–68 kDa (arrow) in lanes 1 and 2, respectively. In panel C, PC2 immunoblots of bovine pituitary and pancreas show an appropriate band of 66–68 kDa (arrow) in lanes 1 and 2, respectively.

endocrine bovine tissues display differential expression of PTP, PC1, and PC2 as demonstrated by immunohistochemistry. Overall, PTP, PC1, and PC2 are colocalized in many neuroendocrine tissues, with some differences in certain tissues such as pituitary, lymphocytes from thymus, and pancreas.

Comparison of Processing Enzyme Proteins by Western Blots

Evaluation of relative levels of processing enzymes was conducted by Western blots analyses of tissue samples. Representative Western blots for PTP, PC1, and PC2 enzymes are shown in Fig. 3. PTP immunoreactivity has been detected as a 33 kDa and in bovine chromaffin granules *(17)*, which is present in rat pituitary and adrenal. It is noted that a slightly lower intensity of PTP in adrenal compared to pituitary was observed in Western blots (Fig. 3A), which is consistent with the relative immunoreactivity of PTP observed by immunohistochemistry in these two tissue regions. Mature PC1 of 66–68 kDa was present in human MDA-MB415 and PC-3 cell lines (Fig. 3B), with slightly greater levels of PC1 in MDA-MB415 compared to PC-3 cells; these results are consistent with immunohistochemistry results. Moreover, mature PC2 of 66–68 kDa was also detected in Western blots of bovine pituitary and pancreas (Fig. 3C), with greater relative levels in pituitary compared to pancreas. These differences in PC2 were also observed by immunohistochemistry. In general, the Western blots demonstrated differential relative levels of processing enzymes that were consistent with differences in immunostaining of fixed cells by immunohistochemistry.

Discussion

Similarities and differences in the distribution of PTP, PC1, and PC2 prohormone processing enzymes were observed in mammalian cell lines and tissues, including those that express PTHrP. These findings support the roles of these enzymes for proteolytic conversion of prohormones into active peptide hormones. PTP represents a more recently characterized cysteine protease for proenkephalin and prohormone processing *(8–10)*. In this study, comparison of the tissue distribution of PTP with that of PC1 and PC2 indicated co-localization of PTP with these subtilisin-like processing enzymes in numerous human, rat, and bovine tissues, suggesting that processing of particular prohormones may involve coordinate participation of several processing enzymes. For example, several human cancer cell lines contain abundant levels of PTP, PC1, and PC2; bovine hypothalamus also possesses prominent levels of all three enzymes. On the other hand, a number of tissues show restricted expression of one or two of these processing enzymes. For example, several human cell lines originating from tumors of the lung, breast, or prostate contain relatively high levels of PTP and PC1, with lower levels of PC2. A B-cell lymphoma cell line, RPMI1788 contained primarily only PTP. Furthermore, bovine thymus contains mainly PTP with minimally detectable PC1 and PC2, whereas, bovine pancreas (islets) contain abundant PC1 and PC2, but not detectable PTP. Significantly, all of these neuroendocrine tissues that produce peptide hormone and peptide neurotransmitters contained at least one of these processing enzymes, which provides support for the roles of PTP, PC1, and PC2 in prohormone and proPTHrP processing. Differential expression of enzyme proteins was also demonstrated by Western blots. Overall, heterogeneity in the profile of processing enzymes present in each cell type suggests differences in relative roles among these enzymes for peptide hormone production in particular cell types.

PTHrP-expressing human cancer cell lines that originated from tumors of breast, lung, and prostate contained the prohormone processing enzymes PTP, PC1, and PC2. Comparison of the relative levels of expression of these three enzymes suggests differential roles in converting pro-PTHrP

into distinct biologically active peptide forms that possess unique biological actions for regulating cell growth, mineral transport, and skeletal cell functions *(4,5)*. In addition, recent studies have demonstrated a role for furin, another member of the subtilisin-like prohormone convertase family, in cleaving proPTHrP *(26)*. The presence of these processing enzymes in tumor cells implicates multiple proteases in the production of distinct PTHrP-related peptides in cancer cells.

In rat and bovine mammalian neuroendocrine tissues, the colocalization of PTP with PC1 and PC2 in adrenal medulla and pituitary are consistent with demonstration of PTP cleavage of proenkephalin that is abundant in the adrenal medulla *(17)*, and POMC that is present in pituitary *(13)*. Importantly, the high levels of PTP, PC1, and PC2 in bovine hypothalamus is consistent with the large number of peptide neurotransmitters and hormones that are synthesized in this brain region *(27)*. The higher levels of PC1 and PC2 immunoreactivity in pancreatic islet cells, compared to PTP, is in agreement with demonstration of PC1 and PC2 as the primary processing proteases for converting pancreatic proinsulin into insulin *(28–30)*. In addition, the presence of PTP, PC1, and PC2 in lymphocytes that contain POMC-derived peptide hormones *(25)* could possibly reflect participation of these proteases in lymphocyte processing of POMC. These processing proteases are, thus, detected in tissues that are abundant in peptide hormones and neurotransmitters.

In summary, the prohormone processing enzymes PTP, PC1, and PC2 are present in a wide variety of neuroendocrine tissues; and they are notably present in PTHrP-expressing human cancer cells originating from tumors of the breast, lung, and prostate. In addition, these processing enzymes are present in cartilage tissue where proPTHrP is processed into biologically active PTHrP peptides. These results are consistent with the hypothesized roles of these enzymes in prohormone processing. Furthermore, differential expression of these enzymes in certain mammalian cells and tissues leads to the prediction of selective roles of these enzymes in processing a particular prohormone in different tissues. It will be of interest in future studies to examine similarities and differences in the specific roles of each of these processing enzymes in processing proPTHrP and other prohormones in normal and pathological conditions of peptide hormone production.

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