

Implications of the subtilisin/kexin-like precursor convertases in the development and function of nervous tissues

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Abstract. Furin, PC1, PC2, and PC5 represent mammalian convertases (PCs) found in endocrine, central and peripheral nervous tissues, which cleave a number of precursors at basic residues normally processed *in vivo*. Typical bonds cleaved by PCs include the pairs Lys-Arg, Arg-Arg and Arg-X-Lys/Arg-Arg. These cleavage sites have been detected following coexpression of each convertase in cell lines together with different precursors as models, including proopiomelanocortin (POMC), proinsulin and proNGF and proBDNF. The presence of PCs and different precursors was revealed by *in situ* hybridization or immunocytochemistry in cultured AtT-20 cells, in the developing CNS, pituitary, and pancreatic islets. In an experimental model of epilepsy in which epileptiform activities were provoked by kainic acid administration, we observed a similar transient expression of furin and PC1 as compared to that of NGF and BDNF. In conclusion, it is proposed that under different stimuli various precursors are activated by a unique cocktail of convertases, each of which either alone or in combination with others acts to process inactive precursors, and thereby playing an important role in development and in the plasticity of the neuronal system.

Review

Key words: prohormone convertase, furin, PC1, PC2, PC5, precursor processing, neuropeptides, neurotrophins, *in situ* hybridization, central and peripheral nervous system, endocrine system

INTRODUCTION

A large number of small, active neuropeptides, hormones, growth factors and proteins are involved in intracellular and extracellular communication. It has been known for almost 3 decades that all these peptides and proteins are initially synthesized as part of larger, biologically-inactive precursors, which when cleaved at specific sites release their active components (Chrétien and Li 1967, Steiner et al. 1967). The precursors are generally synthesized with an amino-terminal signal or leader sequence that is rapidly cleaved off in the endoplasmic reticulum (ER). Removal of the signal sequence is followed by endoproteolytic cleavage by processing enzymes, generally at pairs of basic amino acids. However, not all pairs are cleaved, and certain cleavages take place at single basic residues as well as other sites (Lazure et al. 1983). Yeast pro- α -mating factor (Kurjan and Herskowitz 1982), pro-insulin (Steiner et al. 1992) and pro- ACTH/β -endorphin, also known as proopiomelanocortin (POMC) (Chrétien et al. 1979) are examples of well-studied models of precursor post-translational processing (Fig. 1).

Sequences leading to active peptides may be situated on all sides within the precursor, and a single precursor may contain several peptides with

different bioactivities or many copies of identical bioactive peptides. Thus, cleavage at 4 distinct Lys-Arg sites of yeast pro- α -mating factor results in the release of 4 copies of the active α -factor (Kurjan and Herskowitz 1982, Julius et al. 1984). Active insulin stems from proinsulin fragmentation at its B-chain/C-peptide and A-chain/C peptide junctions at Arg-Arg and Lys-Arg sites (Steiner et al. 1992). In the case of POMC, 7 (or 8 in human) different cleavage sites are possible, but in some tissues only 4 or 5 of them are mostly used. Cleavage at Lys-Arg sites releases ACTH_{1-39} and β -lipotropin. Further cleavage occurs at the GlyLysLysArg¹⁷↓Arg¹⁸Pro site within ACTH_{1-39} to form the ACTH_{1-17} peptide leading to the production of α -melanotropin (α -N-acetyl- $\text{ACTH}_{1-13}\text{NH}_2$) (α -MSH) and ACTH_{18-39} known as corticotropin-like intermediate lobe peptide (CLIP). Finally, cleavage at the Lys-Arg site within β -lipotropin results in the release of γ -lipotropin and β -endorphin₁₋₃₁ and later, after an additional cleavage at LysLys site into β -endorphin₁₋₂₇ (Lazure et al. 1983). Carboxypeptidase E (CPE) removes the exposed C-terminal basic residues and sometimes this trimming reaction is followed by C-terminal amidation by the amidation enzyme (PAM) and/or acetylation of the N-terminus by still undefined acetyltransferases (Eipper et al. 1987).

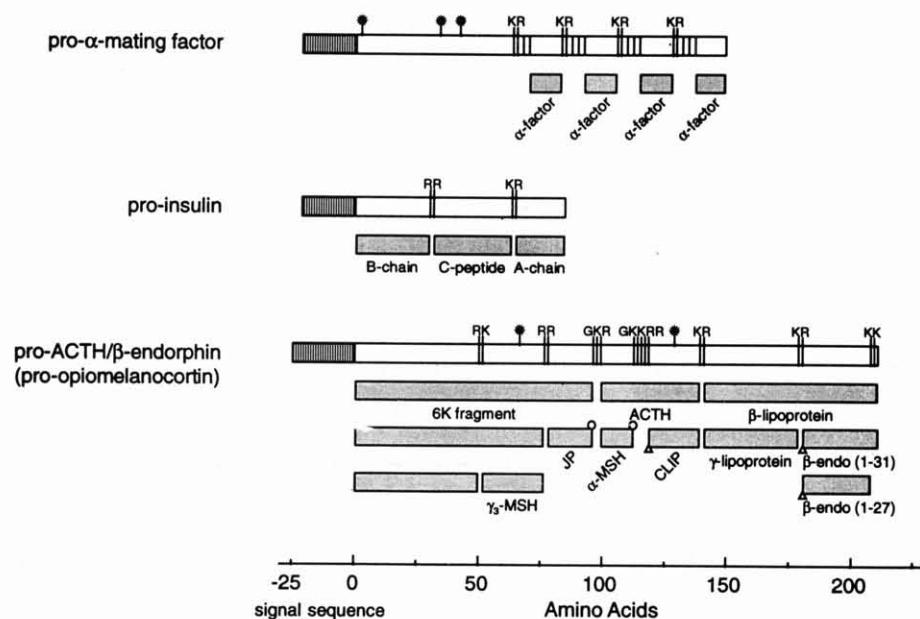


Fig. 1. Structures of three representative precursors: yeast pro- α -mating factor, mammalian proinsulin and proopiomelanocortin, and their products. Single-letter code for amino acids was used: G, glycine; K, lysine; R, arginine. Other symbols are: Δ , α -N-acetylation; $^{\circ}$, α -amidation and $*$, N-linked glycosylation. Abbreviations: JP, joining peptide; CLIP, corticotropin-like intermediate lobe peptide; β -endo, β -endorphin.

The identification of enzymes responsible for the limited endoproteolysis of proproteins remained unresolved until the discovery of Kex2, which mediates the processing of pro- α -mating factor in yeast (Julius et al. 1984, Fuller et al. 1988). Subsequent molecular cloning of enzymes structurally related to Kex2 and to bacterial subtilisins resulted in the definition of 7 mammalian homologues termed subtilisin/kexin-like processing enzymes (PCs) includes several members, of which 6 are produced in the nervous and endocrine systems. These 6 PCs are: furin (Roebroek et al. 1989), PC1 (Seidah et al. 1990) also called PC3 (Smeekens et al. 1991), PC2 (Seidah et al. 1990, Smeekens and Steiner

1990), PC5 (Lusson et al. 1993, Nakagawa et al. 1993), PACE4 (Kiefer et al. 1991) and PC7 (Seidah et al., in press). Of the known convertases, only furin (Van de Ven et al., 1993) and PC5/6-B (Nakagawa et al. 1993), a C-terminally extended isoform of PC5 (Lusson et al. 1993) and PC7 (Seidah et al., in press), contain a C-terminal transmembrane domain. All of them are expressed in neurones (Seidah et al. 1991, Day et al. 1992, Dong et al. 1995, Marcinkiewicz et al. 1993, Schäfer et al. 1993) while furin and PACE4 are also present in glial cells (Day et al. 1992, Dong et al. 1995). The convertase PC4 is only expressed in testicular germ cells (Nakayama et al. 1992, Seidah et al. 1992).

The processing capacity of PCs has usually been studied in cell lines co-infected or co-transfected with a number of substrate precursors. In addition to POMC (Benjannet et al. 1991, Thomas et al. 1991) and proinsulin (Smeekens et al. 1992), representative examples include the processing of prosomatostatin (Galanopoulou et al. 1993), prodynorphin (Dupuy et al. 1994) and proNGF (Bresnahan et al. 1990) to biologically active polypeptides.

Studies on enzymatic cleavage using cellular co-expression procedures provide valuable information on substrate-specificity and indicate the possible preference of some PCs for cells with a regulated secretory pathway. Given a large number of active peptides generated in multiple cell-types of the endocrine and nervous systems, special attention should be paid to cell-specificity and substrate-specificity status in the *in vivo* or at least in the *in situ* context. Accordingly, it is necessary to demonstrate, by co-localization studies at the cellular level, which convertase can affect the *in vivo* maturation of a given precursor. In order to assess the participation of a convertase in the processing of a given precursor we investigated the co-localization of different PCs with different potential substrate-precursors at the cellular level. In the following sections, we will focus on 4 prohormone convertases, namely, furin, PC1, PC2 and PC5. Most of the data presented here have already been published, but some new results are included as well.

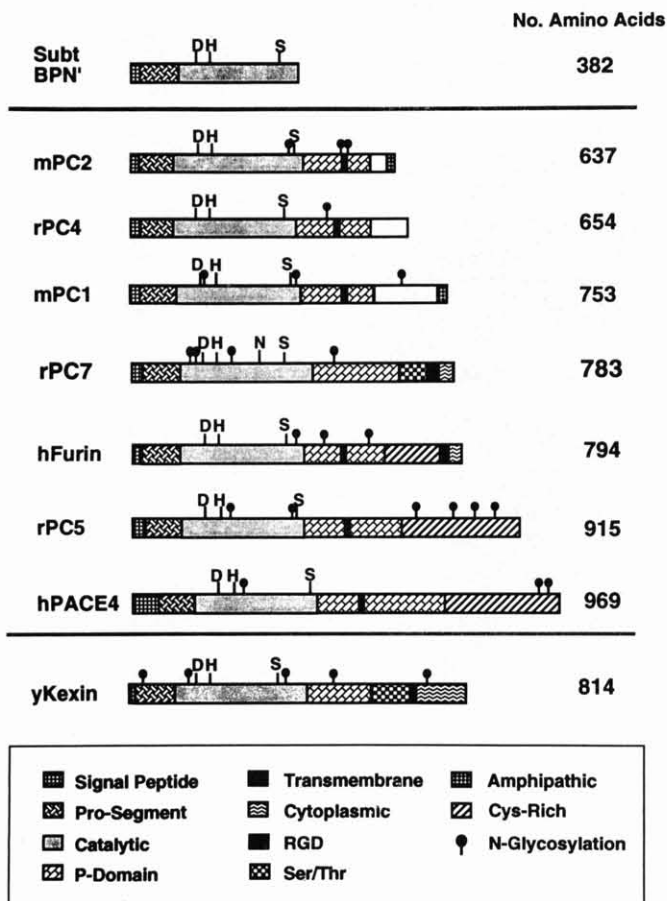


Fig. 2. Schematic representation of the family of mammalian prohormone convertases comparing structural and domain features of each. Also shown are the subtilisin BPN (subt BPN) and yeast Kexin (yKexin).

STEADY-STATE INTRACELLULAR LOCALIZATION OF ENDOPROTEASES

The subcellular localization of PCs has been the subject of several biochemical and immunocytochemical investigations. Xu and Shields (1993) examined the processing of pro-somatostatin in

retrovirally-infected GH₃ cells and deduced that precursor cleavage is initiated in the trans Golgi network (TGN). Furthermore, studies on the biosynthesis and routing of furin introduced to BSC-40 cells by viral infection demonstrated that its staining pattern overlaps with those of several Golgi-associated markers, especially TGN38 (Molloy et al. 1994). A similar observation was made on the intracellular localization of PC1 immunoreactivity in AtT-20 cells. Immunocytochemical staining in

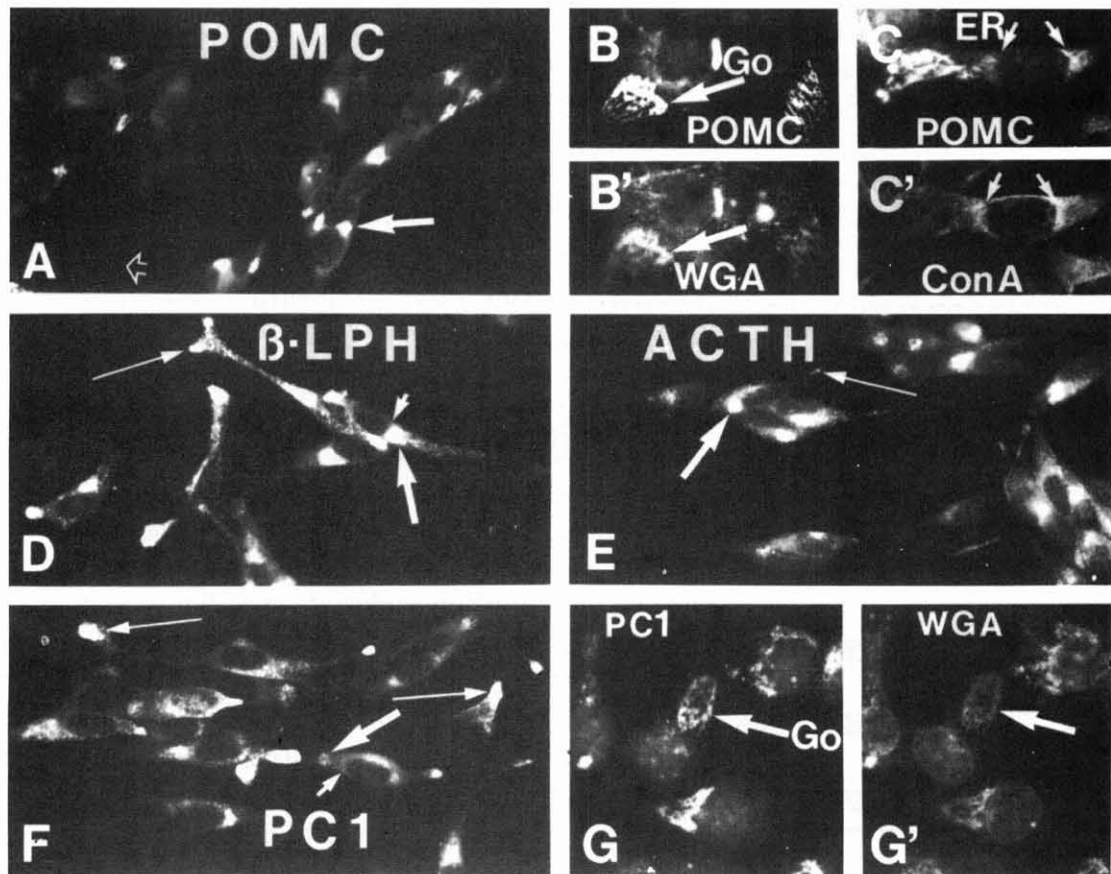


Fig. 3. Intracellular loci of POMC and POMC-derived peptides and PC1 immunoreactivity in AtT-20 cells: colocalization with FITC-labelled WGA and FITC-labelled Concavalin A. (A) paranuclear localization of POMC (arrows), empty arrow indicates cell extensions which are negative. (B and B') comparison of POMC (originally red) with WGA-labelled Golgi apparatus (Go) (originally green), showing that both stainings are superimposable (arrows). (C and C') comparison of POMC with ConA-labelled endoplasmic reticulum (ER) (small arrows). (D) immunoreactive β -LPH seen around cell nuclei (small arrows) at paranuclear position containing Golgi apparatus (arrows) and within tips of cellular extensions (thin arrows). Punctuated staining is also seen all over the cytoplasm, suggesting β -LPH localization within granules. Comparable staining patterns is observed with immunoreactive ACTH (E) and PC1 (F). (G and G') comparison of PC1 with Golgi marker FITC-labelled WGA (arrows). Magnifications: A, D-F = $\times 400$; B, C and G = $\times 700$. The specific antibodies were from rabbit and recognized the intact POMC (Tanaka et al. 1991) and its end-products such as β -Lipotropin and ACTH (antibodies prepared by Dr. M. Lis, Montreal). The antibody to PC1 recognized both its C-terminal (F) (Benjannet et al. 1993) and N-terminal portion (G) (Hornby et al. 1993) of the molecule. The immunofluorescence procedure was as published using TRITC-labeled secondary antibodies (Marcinkiewicz et al. 1993b). FITC-WGA and FITC-Concavalin A were from Molecular Probes, Inc. Eugene, OR, USA).

AtT-20 cells clearly corresponded with Golgi compartments and, in addition, with granules located within cell extensions (Hornby et al. 1993).

Activation of the zymogen form of furin, PC1 and PC5 takes place early in the ER and for PC2 within the TGN and immature granules involving the enzymatic cleavage of their prosegment. This was shown for furin in BSC-40 cells (Molloy et al. 1994), and for both PC1 and PC2 by their biosynthesis in GH₄C₁, β TC3 and Rin m5F cell lines infected with their vaccinia virus recombinants (Benjannet et al. 1993). Finally, PC1 and PC2 immunoreactivity within secretory granules of pancreatic β -cells has been observed by electron microscopy (Malide et al. 1995). Data from our own archives indicate that PC1 is clearly a major convertase produced in AtT-20, and that only extremely low levels of furin, PC2 and PC5 are detectable by immunocytochemistry and *in situ* hybridization (results not shown).

In Figure 3, we compare the immunoreactivity of POMC and 2 intermediate products of its maturation, ACTH and β -LPH with PC1. Although not shown we also examined the immunoreactivity of α -MSH, which was undetectable and β -endorphin, which resembled that of β -LPH. In most cases, superimposable patterns of intracellular distribution are evident: POMC immunoreactivity partially overlaps with PC1 within presumptive ER and Golgi structures. Furthermore, co-localization of PC1 with ER and Go markers, such as Fluorescein-labelled Concanavalin A (ConA) and Fluorescein-labelled WGA, respectively (Virtanen et al. 1980, Lippincott-Schwartz et al. 1989, David-Pfeuty and Nouvian-Dooghe 1990), has led to the detection of its intracellular loci. Thus, minor PC1 immunoreactivity found around cell nuclei superimposable with ConA marker is assigned to the ER, whereas the major site of PC1 immunoreactivity at para- or orthonuclear positions corresponds to Go cisternae. This has resulted in the conclusion that steady state intracellular PC1 localization in AtT-20 cells agrees well with patterns of POMC, POMC intermediate (ACTH, β -lipotropin) and end-products (β -endorphins), and that this evidence is in close accord with

previous biochemical data on PC1-mediated POMC processing (Benjannet et al. 1991, Thomas et al. 1991). Interestingly, this result also suggests that at low furin, PC2 and PC5 concentrations, no α -MSH can be detected in AtT-20 cells, indicating that below some critical enzyme density, especially of PC2, processing of ACTH₁₋₃₉ to ACTH₁₋₁₇ cannot occur.

DISTRIBUTION IN NERVOUS AND ENDOCRINE TISSUES

Tissue-distribution data on PCs, obtained by Northern blot analysis, *in situ* hybridization and immunocytochemistry have been reported in numerous publications. They demonstrate a unique distribution of each enzyme, especially in the CNS, pituitary and some peripheral organs, including the heart and lungs, as well as endocrine organs such as the thyroid, adrenals, gut and gonads. Table I summarizes some data on the tissue-distribution of furin, PC1, PC2 and PC5. Whereas mRNA for furin is ubiquitously present in most tissues and cells, PC1, PC2 and PC5 seem to be less widespread, emphasizing their specialization. *In situ* hybridization produces usually a characteristic pattern for each convertase, or mosaic with some hot spots. From comparisons of individual mosaics, it can be deduced that multiple centers contain more than one convertase, as for example in the CNS, cerebral cortex, hippocampus, several hypothalamic nuclei, peripheral nervous system (PNS), the trigeminal and spinal nuclei, the anterior lobe of the hypophysis, C-cells of the thyroid gland, pancreatic β -cells, and others. In some cases, just based on the histochemical pattern it is possible to predict the potential substrate(s) for a given convertase. This is true, for instance with PC1 and PC2 within pancreatic β -cells (Marcinkiewicz et al. 1994, Malide et al. 1995) which are known to synthesize proinsulin and for PC2 in the intermediate lobe of the pituitary (Seidah et al. 1990, 1991, Marcinkiewicz et al. 1993) producing α -MSH. More complex studies have however, been necessary to define the status of furin, PC1 and PC2 in adenohypophyseal cor-

TABLE I

Tissue distribution of prohormone convertases				
Tissue	furin	PC1	PC2	PC5
Nervous system*:				
neurons	hh ¹ im ²	hh1,3-6 im ⁵	hh ^{1,3-6} im ⁵	hh ⁷
glial cells	hh ¹			hh7
choroid plexus cells	hh ¹ im ^{2,8}			
ependyma	hh ¹ im ^{2,8}			
Endocrine system:				
hypophysis	hh ¹	hh ^{3,4} im ^{5,9}	hh ^{3,4} im ^{5,9}	hh ⁷
thyroid gland	hh ¹⁰	hh10	hh ¹⁰	hh10
parathyroid	hh ¹⁰			
pancreas		hh ¹¹ im ¹¹⁻¹⁴	hh ¹¹ im ¹¹⁻¹⁴	
gut		im ¹³	im13	hh ¹⁵
adrenals	hh ¹⁶	hh ¹⁶	hh ¹⁶	hh ¹⁶
Exocrine tissues:				
lacrimal gland	hh ¹⁵			
submaxillary gland	hh ¹⁵			
Bowmans glands	hh ¹⁵			
Cardiovascular system	hh ^{17,18}	hh ^{17,18}	hh ^{17,18}	hh17,18

Abbreviations: hh, hybridization histochemistry; im, immunocytochemistry. Data are from the following sources: (1) Day et al. 1993, (2) Marcinkiewicz et al., unpublished observations, (3) Seidah et al. 1990, (4) Seidah et al. 1991, (5) Marcinkiewicz et al. 1993a, (6) Schäfer et al. 1992, (7) Dong et al. 1995, (8) Gee et al. 1993, (9) Marcinkiewicz et al. 1993b, (10) Hendy et al. 1995, (11) Marcinkiewicz et al. 1994, (12) Smeekens et al. 1992, (13) Scopsi et al. 1995, (14) Malide et al. 1995, (15) Marcinkiewicz et al. this work, (16) Seidah et al. 1993; (17) Beaubien et al. 1995, (18) Zheng et al., 1994; (*) detailed comparative distribution of PCs in the central nervous system is reviewed by Chrétien et al., in press.

ticotrophs because these cells change properties during ontogeny.

It is noteworthy that only the transient presence of PC2 was observed in corticotrophs from newborn mice. Interestingly, PC2 expression coincided with short-time span production of α -MSH in the same corticotrophs, suggesting that at a high level of this particular convertase, ACTH₁₋₃₉ processing into ACTH₁₋₁₇ and CLIP takes place (Marcinkiewicz et al. 1993b). Thus, it has become clear that analysis of temporal expression patterns should provide insights into physiologically-relevant functions of PCs, such as the production of α -MSH which plays an important role in development. Studies on pituitary (Marcinkiewicz et al. 1993b, Zheng et al.

1994), pancreatic (Marcinkiewicz et al. 1994) and CNS ontogeny (Marcinkiewicz et al. 1993a, Zheng et al. 1994) have directed our attention to the plasticity of the PCs expression in general. In the nervous system this plasticity may also be important for growth, synapse organization, as well as for the biosynthesis of active mediators and neurotrophic factors. Developmental events may also be reproduced in adults in processes involving neuroprotection and neuronal regeneration. With this interest in mind, we further investigated the expression of PCs during ontogeny in the embryo, in postnatal mice, and in adults, following the cerebral insults with epileptogenic drugs.

ONTOGENY

To test whether PCs are implicated in the processes of central and peripheral nerve development, we examined their expression in the embryo. As in the rat (Zheng et al. 1994) we found that in mice furin was expressed in a number of non-neuronal structures, while PC1 and PC2 were confined to nervous and endocrine tissues. PC5 expression was restricted to a few structures containing both neuronal and non-neuronal cells.

According to Zheng et al. (1994), furin is already expressed in both endoderms and mesoderms in the

primitive streak stage on embryonic day 7 (e7), while in embryonic ectoderms, from which the nervous system is derived its mRNA transcripts are undetectable. The overall expression pattern outside the presumptive nervous system is maintained until e10, when a higher furin expression is observed in the cardiovascular and liver primordia, while cephalic regions only express low to medium levels of transcripts (Zheng et al. 1994). In the rat, furin is transiently expressed in the Rathkes pouch region from e13 to e18 (Zheng et al. 1994). The nervous system remains negative for furin until e18 in the rat (Zheng et al. 1994) or e16 in mouse (our

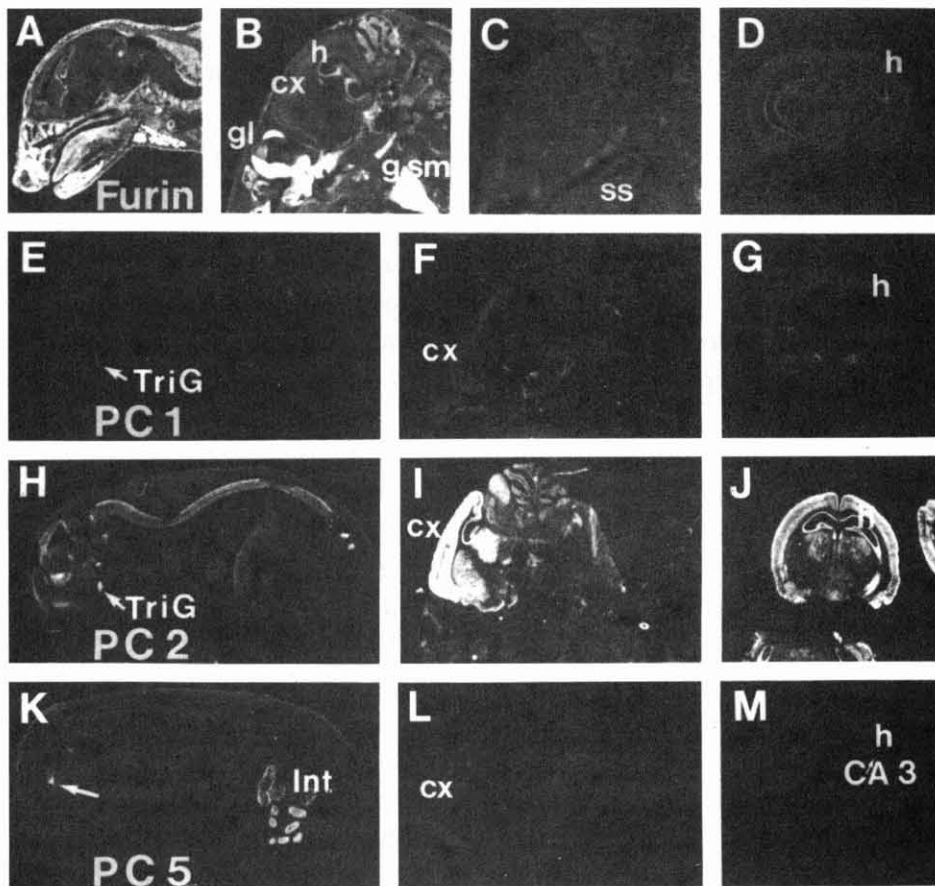


Fig. 4. mRNA for PCs in the embryonic, post-natal and adult CNS. A presence of mRNA for furin (A-D); PC1 (E-G); PC2 (H-J) and PC5 (K-M) is shown by *in situ* hybridization by day 18 (A,E,H,K), postnatal day 16 (B,C,F,I,L) and adult (D,G,J,M). cRNA 35 S-UTP labelled antisense probes with were used for hybridization histochemistry as described (Marcinkiewicz et al. 1993b). Control with the sense furin riboprobe is in (C). Autoradiography was produced using X-ray film (Kodak). Widespread labeling is evident for furin, encompassing several exocrine tissues, such as the lacrimal gland (gl) and submaxillary gland (gsm). PC1 and PC2 show labelling within CNS and PNS, including the trigeminal nucleus (TriG). Discreet PC5 labelling is seen in embryonic and postnatal brain (medium arrow), while in adult it is seen in the deep cortical layers and in CA3 area of the hippocampus. Abbreviations are: CA3, area 3 of the hippocampal formation; cx, cerebral cortex; gl, lacrimal gland; gsm, submaxilar gland; Int, intestine; h, hippocampus; TriG, trigeminal ganglion. Magnification: x4.

unpublished data), except for the choroid plexus and some ependymal cells. As shown in Fig. 4, cerebral expression of furin occurs in brain on embryonic day 18 (e18) and the postnatal period including day 16 (p16) it becomes widely expressed, encompassing several regions such as the hippocampus, cerebral cortex, cerebellar Purkinje cells and olfactory bulb. Nevertheless, furin expression in cerebral tissues, which appears to be maximal postnatally (Fig. 4B) is still much lower in comparison to that found in some peripheral tissues, including the lacrimal gland, Bowmans glands in olfactory turbinate tissues, liver and stomach mucosa. Finally, appreciable levels of hybridization can be seen in chondrification centers, including in chondrocytes and their immediate precursors.

PC1 and PC2 mRNAs are already evident by mid-gestation (e10 and e11) in pancreatic primordia (Marcinkiewicz et al. 1994). Expression in pituitary primordia seems to occur 1 or 2 days later and it is similar for neuronal tissues. The onset of PC1 and PC2 expression in the CNS and PNS coincides with an appearance of postmitotic neurones. During the later periods of embryonic development (e16-e19), the expression of PC1 and PC2 reaches appreciable levels both in intensity and extent in the future brain, spinal cord and cranial ganglia including the trigeminal ganglion (TriG) (Fig. 4) and spinal ganglia (data not shown). PC1 expression seems to increase maximally by adulthood, while higher levels of PC2 expression are found postnatally in the brain (Marcinkiewicz et al. 1993a) and in the pituitary gland (Marcinkiewicz et al. 1993b).

In early and later ontogeny stages, PC5 expression seems to be associated with non-neuronal tissues, including connective tissue around the cardiovascular apparatus and ossification centres. In later gestation stages a strong PC5 hybridization signal can be detected in developing intestine tissue. Only a few telencephalic and mesencephalic centres in the spinal cord contain cells expressing PC5. In regard to the intensity and extent of the hybridization signal in the CNS, PC5 attains a maximum of expression in adulthood.

In summary, like PC5 the expression of PC1 in a number of the neuronal structures increases pro-

gressively until adulthood. Furin and PC2 show a widespread distribution throughout the central and peripheral nervous system and are elevated postnatally within time period p16-p22. Unlike PC1 and PC2, hybridization hot spots for both furin and PC5 are found outside the nervous and endocrine tissues. The expression of all PCs studied in the nervous system so far seems to be associated rather with postmitotic than with premitotic neurones. Therefore, their function may involve the consolidation of neuronal structures during later gestation period. Vis à vis the ontogeny of non-neuronal peripheral tissues, PC1 and PC2 can mainly be found within endocrine centres, which indicates that they play a role in the biosynthesis of peptides and hormones. Both furin, which presents a widespread expression pattern throughout all ages and tissues, and PC5 which has more restricted expression sites, seem to be expressed during growth in a number of organs and in the vasculature, chondroification as well as ossification centres. Specifically high levels of expression in some exocrine tissues, such as the olfactory neuroepithelium, lacrimal gland and salivary gland, suggest that furin may be one of the molecular players involved in the regulation of hydromineral balance in these tissues. Finally, it is evident that PCs are present in different combinations in CNS and PNS at the particular stage of increased neuropeptides and neurotrophins production. Therefore, it is likely that they may be implicated in their post-translational processing. Biochemical studies on processing of proNGF and proBDNF addressed this point and showed that under experimental cellular co-expression conditions, more than one convertase can activate these precursors. The challenge is to establish which candidate PC is responsible for NGF and BDNF and other neurotrophins processing *in vivo*.

PRO-NGF AND PRO-BDNF PROCESSING

Since publication of proNGF cDNA structure (Scott et al. 1983), four other members of the family have been identified and their cDNAs cloned: brain-derived neurotrophic factor (BDNF) (Leibrock et al.

1989), neurotrophin-3 (NT3) (Ernfors et al. 1990, Maisonnier et al. 1990), neurotrophin-4/5 (NT-4/5) (Berkemeier et al. 1991), and neurotrophin-6 (NT-6) (Gotz et al. 1994). Sequence data predict that all neurotrophins are generated from 31–35 kDa precursors that contain at their amino termini, hydrophobic signal peptides followed by pro-regions containing sequences of contiguous basic amino acids. Intracellular cleavage of the pro-neurotrophins to produce active growth factors occurs following pairs of basic amino acids of the type I precursor motif (Seidah 1995) Arg-X-(Lys/Arg)-Arg↓, where X=Ser, Val or Arg for proNGF/proNT-4/5, proBDNF/proNT-6 and proNT3, respectively.

Biochemical analysis of the processing of proNGF by the five possible candidate convertases revealed that furin, PACE4 and PC5/6-B are the best proNGF processing enzymes in both constitutively secreting and regulated cells (Seidah et al. 1995). Similarly, biochemical analysis of the cellular processing of proBDNF, demonstrated that among the known mammalian serine proteinases of the subtilisin/kexin family furin, PACE4 and PC1 represent the best convertases of proBDNF (Seidah et al. 1994). Therefore, the sum of the present knowledge suggests that more than one convertase

can process the neurotrophins, but that furin is the most efficient in processing these precursors.

Aside from cleavage specificity the cognate convertase(s) must also be correctly co-localized and possibly co-regulated with their substrate within either the trans-Golgi Network (TGN) or in secretory granules. Accordingly, studies on the co-localization of the neurotrophin mRNAs with those of the convertases and their co-regulation revealed that more than one convertase can co-localize with the neurotrophins (Marcinkiewicz et al. 1994). Thus, biochemical data fit well with the co-localization results which demonstrate that in the brain NGF/BDNF and furin mRNA coincide, and that BDNF/PC1 are also co-ordinately upregulated following kainic acid treatment. The mRNA levels of furin and PC1, but not of PC2 and PC5, increase in hippocampal structures 3 h following kainic acid administration (Meyer et al. submitted). Moreover, the observed upregulation appears to be parallel to that of NGF and BDNF mRNAs which were elevated (NGF) or maximal (BDNF) 3 h after stimulus. The results suggest a potential implication of furin and/or PC1 in proNGF and proBDNF processing during epileptiform activity. Since neither co-habitation or co-regulation of PACE4 or PC5/6-B with

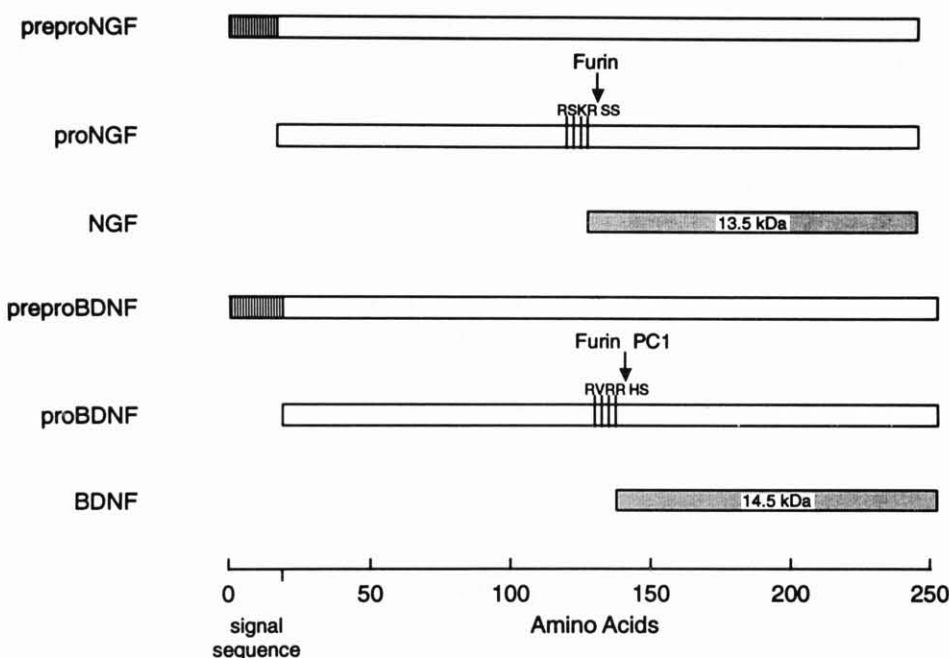


Fig. 5. Structures of NGF and BDNF precursors and their. Based on available data the *in vivo* processing of proNGF may involve a cleavage at Arg-Ser-Lys-ArgSer-Ser site by furin, while both furin and PC1 would be implicated in the processing of proBDNF at Arg-Val-Arg-Arg↓His-Ser site (Seidah et al. 1994).

either BDNF or NGF is evident following hippocampal seizures, and in accordance with the biosynthetic data (Seidah et al. 1995) and the *in situ* hybridization analysis (this work, Marcinkiewicz et al. 1994, Meyer et al., submitted) we conclude that in brain, furin and furin/PC1 are the best candidate proNGF and proBDNF convertases, respectively (Fig. 5).

CONCLUSIONS

The results presented here provide a framework for understanding the relationships between prohormone convertases and multiple peptides, hormones and growth factors in *in vivo* conditions. The events involving PCs include proteolytic cleavage of precursors at specific sites which may vary along ontogeny and may come into view in different physiological situations, when biosynthesis of peptides increases i.e. epileptic seizures.

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