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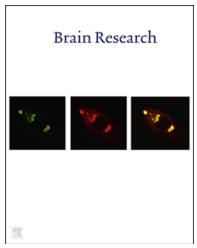

Research Report

Prolyl carboxypeptidase mRNA expression in the mouse brain

Jin Kwon Jeong[a,b], *Sabrina Diano*[a,b,c,d,*]

[a]*Program in Integrative Cell Signaling and Neurobiology of Metabolism, Yale University School of Medicine, New Haven, CT, USA*
[b]*Department of Ob/Gyn & Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA*
[c]*Department of Neurobiology, Yale University School of Medicine, New Haven, CT, USA*
[d]*Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT, USA*

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ABSTRACT

Prolyl carboxypeptidase (PRCP), a serine protease, is widely expressed in the body including liver, lung, kidney and brain, with a variety of known substrates such as plasma prekallikrein, bradykinin, angiotensins II and III, and α-MSH, suggesting its role in the processing of tissue-specific substrates. In the brain, PRCP has been shown to inactivate hypothalamic α-MSH, thus modulating melanocortin signaling in the control of energy metabolism. While its expression pattern has been reported in the hypothalamus, little is known on the distribution of PRCP throughout the mouse brain. This study was undertaken to determine PRCP expression in the mouse brain. Radioactive in situ hybridization was performed to determine endogenous PRCP mRNA expression. In addition, using a gene-trap mouse model for PRCP deletion, X-gal staining was performed to further determine PRCP distribution. Results from both approaches showed that *PRCP* gene is broadly expressed in the brain.

Abbreviations: PRCP: CNS, Central Nervous System; Prolyl carboxypeptidase, α–MSH: alpha-melanocyte stimulating hormone; MC3R, melanocortin 3 receptor; MC4R, melanocortin 4 receptor; Lv, lateral ventricle; 3v, third ventricle; 4v, fourth ventricle; HP, hippocampus; HYP, hypothalamus; Thal, Thalamus; Amyg, amygdala; Cb, cerebellum; Cing Ctx, cingulate cortex; VTA, ventral tegmental area; NTS, nucleus of solitarius tract; DMV, dorsal motor nucleus of the vagus; f, fornix; ec, external capsule; Pir ctx, piriform cortex; ac, anterior commissure; MPO, medial preoptic nucleus; LPO, lateral preoptic area; PVN, paraventricular nucleus of hypothalamus; ARC, arcuate nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus; DMH, dorsomedial hypothalamic nucleus; LH, lateral hypothalamus; SN, substantia nigra; BLA, basolateral amygdala; DG, dentate gyrus; Pir Ctx, piriform cortex; MPO, medial preoptic area; LPO, lateral preoptic area; LSN, lateral septal nucleus; SO, supraoptic nucleus; PV, paraventricular nucleus of the thalamus; Xi, xiphoid thalamic nucleus; MHb, medial habenular nucleus; VM, ventromedial nucleus of the thalamus; D3v, dorsal third ventricle; SN, substantia nigra; ZI, zona incerta; MM, mammillary body; VTA, ventrotegmental area; CA, cerebral aqueduct; Pn, pontine nuclei; PnO, pontine reticular nucleus; RtTg, reticulotegmental nucleus of the pons; LVe, lateral vestibular nucleus; MVe, medial vestibular nucleus; Pr, prepositus nucleus; Sp5, spinal trigeminal nucleus; Gi, gigantocellular reticular nucleus; ECu, external cuneate nucleus

*Corresponding author at: Department of Ob/Gyn & Reproductive Sciences, Yale University School of Medicine, PO Box 208063, New Haven, CT 06520-208063, USA. Fax: +1 203 7854713.
E-mail address: sabrina.diano@yale.edu (S. Diano).

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1. Introduction

Prolyl carboxypeptidase (PRCP) is an enzyme of the carboxypeptidase (CPs) family which contains a serine residue in the active center, essential for catalytic activity (Abeywickrema et al., 2010; Soisson et al., 2010). PRCP cleaves only short peptides with a penultimate proline residue (Kumamoto et al., 1981).

PRCP was discovered over 40 years ago from studies of bradykinin metabolism in kidney (Yang et al., 1968). Since angiotensin II has the same C-terminus of bradykinin, the enzyme was named angiotensinase C. However, when its ability to cleave a variety of Pro-X bonds was shown, it was renamed PRCP.

PRCP (Yang et al., 1968, 1970) is a single chain protein of approximately 58 KDa. The C-terminal sequence contains an interesting "serine repeat" in which Ser is repeated as the 26th residue 6 out of 9 times (Tan et al., 1993). When the Ser residues are aligned to yield 26 residue repeats, the positions of some of the other residues also fall into a pattern. The significance of this repeat region is not clear, but it might be involved in the maintenance of a secondary or tertiary structural motif or in the formation of the homodimer. The gene encoding human PRCP is on chromosome 11, while in the mouse it is on chromosome 7.

PRCP has an acidic pH optima (=5.0) when hydrolyzing short synthetic peptide substrates (Odya et al., 1978; Jackman et al., 1990; Tan et al., 1993), but it has been found that it retains significant activity at neutral pH range. Indeed, at a physiological pH, α-melanocyte stimulating hormone (α–MSH) is a better substrate for PRCP than angiotensin II (Wallingford et al., 2009).

PRCP activity has been detected in a variety of cells and organs both in rodents and human (Kumamoto et al., 1981; Skidgel et al., 1981; Tan et al., 1993). Within the cell, it has been localized in lysosomes (Kumamoto et al., 1981; Skidgel et al., 1981; Jackman et al., 1995). However, it has also been found to be released in response to stimulation appearing in extracellular media or biological fluids (Yang et al., 1960; Miller et al., 1991). In mice, like humans, PRCP is expressed in peripheral tissues such as kidney, liver, heart and spleen. In addition, PRCP is also expressed in the Central Nervous System (CNS; Wallingford et al., 2009). Specifically, within the CNS, recent studies have shown PRCP expression and regulation in the hypothalamus (Wallingford et al., 2009; Jeong et al., 2012a, 2012b, 2013).

The distribution pattern of PRCP in the CNS has not been explored in details. Thus, our study was undertaken to determine the expression pattern of PRCP within the CNS.

2. Results

Radioactive in situ hybridization using a riboprobe specific to PRCP mRNA was performed in adult C57Bl6 mouse brains (Fig. 1). In addition, PRCP expression pattern was studied using X-gal staining in PRCP transgenic mice in which trapped PRCP gene contained an insertion that had the following regions in its vector (pGT1TM) from 5′ to 3′ called SA (splice acceptor), CD4-TM, and a lacZ reporter (Skarners et al., 1995; Wallingford et al., 2009; Figs. 2 and 3). The results obtained from both methodologies were overlapping. PRCP gene expression was observed throughout the mouse brain with a differential expression pattern (Table 1).

2.1. *Expression of PRCP in the telencephalon*

The overall localization and expression levels of PRCP mRNA in adult mice brain is summarized in Table 1.

The greatest levels of both endogenous and transgene signals were detected in the cerebral cortex with very strong labeling in the cingulate (Cing Ctx; Fig. 1C,F,O, Fig. 2A–F, Fig. 3A) and piriform cortex (Pir ctx; Fig. 1B,F,Q, Fig. 2A–F, Fig. 3C). Other areas of the cortex showed moderate signal intensity. Within the limbic system, strong signal was detected in the hippocampus and in the amygdaloid complex. Both X-gal and silver grain density were weaker in the rostral portion of the hippocampus but became stronger in the caudal portion. Within the hippocampus, both signals were strong in all regions of the Ammon's horn and in the dentate gyrus (Fig. 1B,C,F,G,P, Fig. 2C–F, Fig. 3B). In the amygdala, high intensity of the signal was detected within the the basolateral- (Fig. 1F,N, Fig. 2D and Fig. 3C) and central-amygdaloid nucleus, while moderate signals were observed in the anterior-amygdaloid nucleus (BLA; Fig. 2D and Fig. 3C). PRCP labeling was also detected within the septum pellucidum, specifically the lateral septal nucleus (LSN; Fig. 2A).

2.2. *Expression of PRCP in the diencephalon*

Strong PRCP expression was detected within the thalamus. Specifically, the paraventricular thalamic nucleus (PV; Fig. 1F, Fig. 2B–D, Fig. 3G) and the Xiphoid thalamic nucleus (Xi; Fig. 2C) showed the stronger staining within the thalamic formation.

In the epithalanus, the habenular nucleus (MHb) showed significant staining (Fig. 2D, Fig. 3H).

Within the hypothalamus, moderate to weak levels of both endogenous PRCP mRNA and X-gal staining were detected. Specifically, both X-gal staining and in situ hybridization for PRCP mRNA show moderate signals in the medial (MPO) and lateral preoptic area (LPO; Fig. 1I, Fig. 2A and Fig. 3D), in the paraventricular nucleus (PVN; Fig. 1B, J; Fig. 2C and Fig. 3F), in the supraoptic nucleus (SO; Fig. 2B C and Fig. 3E), in the dorsomedial hypothalamic area (DMH; Figs. 1C, K, Fig. 2D and Fig. 3J), in the lateral hypothalamus (LH; Figs. 1F,L, Fig. 2D and Fig. 3I,J) and in the arcuate nucleus (ARC; Fig. 1C,K, Fig. 2D and Fig. 3I). On the other hand, while by in situ hybridization, moderate signals for PRCP mRNA expression was detected in the ventromedial hypothalamus (VMH; Fig. 1C,K), a very weak and/or inconsistent staining by X-gal staining was detected (Fig. 2D and Fig. 3I). Similar discrepancy was found in the bed nucleus of stria terminalis (BST), in which only hybridization signal was detectable, but not X-gal staining (data not shown).

2.3. *PRCP expression in the mesencephalon and rhombencephalon*

In the midbrain, PRCP labeling was detected in the zona incerta (ZI; Fig. 2E) and the ventral tegmental area (VTA; Fig. 1M, Fig. 2F, Fig. 3K).