Prohormone converting endopeptidase is encoded by a eukaryotic expression vector under the control of the human cytomegalovirus promoter. This vector encodes for the antibiotic resistance gene neomycin as a selectable marker. Transfection was carried out using the calcium phosphate precipitation method and selection of resistant cells was achieved using G418 sulphate at 900 µg/ml in the grow media. Clones were picked up at random and screened for the expression of PC1 or PC2 enzymes by immunofluorescence using specific antibodies to PC1 and PC2 (kindly provided by Dr. I. Lindberg, Louisiana State University, USA).

In order to investigate if PC1 or PC2 are capable of correctly cleaving proCRH within CHO-K1 cells we transfected an expression plasmid containing the PC1 cDNA and PC2 cDNAs into stably transfected CHO-K1 cells already expressing proCRH (clone 1/22) [2]. Expression vectors carrying PC1 or PC2 cDNAs were constructed by ligating the 2,615 bp and 2,223 bp fragments of PCI and PC2 cDNAs respectively, into the PEE6hCMV-ne, the subtilisin family of serine proteases, PC2 might be the candidates for the endoproteolytic processing of PCI and PC2 cDNAs respectively, into the PEE6hCMV-ne.

Hormones and neuropeptides are generally synthesized as large precursor molecules: i.e. prohormones and propeptides which undergo endopeptidase to produce biologically active peptides. Corticotrophin releasing hormone (CRH) is a 41 aminoacid peptide which plays a major role in the regulation of the endocrine response to stress. CRH(1-41) is generated after endopeptidolytic cleavage at pairs of basic aminoacid residues from its precursor molecule: proCRH [1]. Two enzymes which belong to the subtilisin family of serine proteases, PCI (also called PC3) and PC2 might be the candidates for the endoproteolytic processing of proCRH, due to their cleavage specificities and their expression in tissues of endocrine-neuroendocrine origin.

In order to investigate if PC1 or PC2 are capable of correctly cleaving proCRH within CHO-K1 cells we transfected an expression plasmid containing the PC1 cDNA and PC2 cDNA into stably transfected CHO-K1 cells already expressing proCRH (clone 1/22) [2]. Expression vectors carrying PC1 or PC2 cDNAs were constructed by ligating the 2,615 bp and 2,223 bp fragments of PCI and PC2 cDNAs respectively, into the PEE6hCMV-ne, the subtilisin family of serine proteases, PC2 might be the candidates for the endoproteolytic processing of PCI and PC2 cDNAs respectively, into the PEE6hCMV-ne.

Our results indicate that CRH and PCI or PC2 are targeted into the secretory pathway within transfected CHO-K1 cells. To determine if PC1 and PC2 are able to cleave proCRH we subjected cell extracts and secreted medium from 1/22/PC1 and MT-PC2/CRH cells to gel exclusion chromatography by using Sephadex G50. The chromatographic profile from cells co-expressing proCRH and PC2 (MT-PC2/CRH) showed that they store and secrete the intact precursor and smaller forms of CRH. Cells co-expressing proCRH and PCI store and secrete uncleaved proCRH.

The immunoreactive (IR) staining pattern of CRH showed a reticular cytoplasmic distribution as well as nuclear foci within both stably transfected CHO-K1 cells co-expressing CRH and PCI (1/22/PC1 clone) (Fig 1) or CRH and PC2 (MT-PC2/CRH clone) (Fig 2). Double labelling immunofluorescence studies using antibodies against resident proteins of the endoplasmic reticulum and Golgi apparatus (TGN38 protein) also showed co-localization of IR-CRH within these organelles (data not shown). PC1 in cells co-expressing CRH and PCI (1/22/PC1 clone) (Fig 1) and IR-PC2 in cells co-expressing CRH and PC2 (Fig. 2) also showed the classic reticular staining pattern corresponding to the endoplasmic reticulum. Our results indicate that CRH and PCI or PC2 are targeted to the secretory pathway within transfected CHO-K1 cells.

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