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Original Article

Immunocytochemical Localization of Peptidylglycine Alpha-amidating Monooxygenase Enzymes (PAM) in Human Endocrine Pancreas[1]

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We studied the distribution of the enzymes that are involved in the post-translational alpha-amidation of regulatory peptides in human endocrine pancreas, using immunocytochemical methods for light and electron microscopy. Immunoreactivity for the two enzymes involved, peptidylglycine alpha-hydroxylating monooxygenase (PHM) and peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL), was located in the periphery of the islets of Langerhans and in ductal endocrine cells. Staining of reverse-face serial sections demonstrated that these immunoreactivities co-localize with glucagon but not with pancreatic polypeptide (PP), insulin, or somatostatin. Double immunogold staining for electron microscopy confirmed the previous results and revealed a different localization for each enzyme inside the secretory granule: PHM is present in the central core of the glucagon-containing granules, whereas PAL is predominantly located near the granule membrane. The existence of an amidated peptide, GLP1, in the A-cells explains the presence of peptidylglycine alpha-amidating monooxygenase enzymes (PAM) in these cells. The absence of the enzymes in the PP-cells raises the possibility that a different form of amidating enzyme may be involved in the post-translational processing of this peptide. (*J Histochem Cytochem 41:375–380, 1993*)

KEY WORDS: PAM; PHM; PAL; Human pancreatic islets; Glucagon; PP; Insulin; Somatostatin; Immunocytochemistry; Double immunogold staining.

Introduction

Bioactive peptides frequently bear a carboxy-terminal alpha-amide as the result of a post-translational process essential for biological activity (May et al., 1990). The enzymes responsible for this modification are collectively known as peptidyl-glycine alpha-amidating monooxygenase or PAM (E.C. 1.14.17.3).

Human PAM precursor has been cloned from thyroid carcinoma cells (Glauder et al., 1990). As in other mammalian species, the gene encodes two proteins, both of them involved in the alpha-amidation of peptides: peptidylglycine alpha-hydroxylating monooxygenase (PHM) catalyzes the conversion of glycine-extended pro-peptides into peptidyl-alpha-hydroxyglycine intermediates. The second step is carried out by the peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL) (Eipper et al., 1991).

PAM enzymatic activity has been biochemically described in a variety of endocrine (e.g., Birnbaum et al., 1989a; Markosian et al., 1989; Sheldrick and Flint, 1989; Katopodis and May, 1988; Glembotski, 1985) and non-endocrine (e.g., Rhodes et al., 1990; Birnbaum et al., 1989b; Von Zastrow et al., 1986; Eipper et al., 1985; Mains et al., 1985) tissues. Precise immunocytochemical localization of the involved cells has been carried out only in heart (Eipper et al., 1988), pituitary (May et al., 1990), and central nervous system (Schafer et al., 1992; Rhodes et al., 1990).

There is biochemical evidence of PAM activity in the islets of Langerhans of fish and rats: a cytochrome b561 has been described in the membrane of the secretory granules as a component of the ascorbate-dependent PAM system in the anglerfish (Mackin et al., 1986) and, in the same animal, the islet secretory granule lysates show PAM activity with the same requirements as its mammalian counterpart: copper, ascorbate, and molecular oxygen (Mackin et al., 1987). The presence of the amidating enzymes in rat pancreatic islet culture (Scharfmann et al., 1988), as well as the expression of PAM mRNA (Maltese et al., 1989), has also been reported.

Although these studies show PAM activity to occur in many tissues, there is little morphological evidence about which cells contain the enzymes, whether the enzymes act in vivo as a single bifunctional protein or are cleaved to act separately, whether they act

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