Localization of Amidating Enzymes (PAM) in Rat Gastrointestinal Tract

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We studied the distribution of the two enzymes involved in post-translational C-terminal α-amidation of regulatory peptides in rat digestive tract, using immunocytochemical methods and in situ hybridization techniques. The enzymes were located in most of the fibers and neurons of the myenteric and submucous plexus throughout the entire digestive tract and in endocrine cells of the stomach and colon. Staining of reverse-face serial sections demonstrated that the enzymes in endocrine cells of the stomach co-localized with gastrin in the bottom of the gastric glands. Some gastrin-immunoreactive cells near the neck of the gland were negative for PAM, suggesting that amidation takes place only in the more mature cells. In the colon all cells immunoreactive for glucagon and GLP1 were also positive for peptidylglycine α-hydroxylation monoxygenase (PHM) but not for peptidyl-α-hydroxyglycine α-amidating lyase (PAL). The absence of immunoreactivity for the amidating enzymes in endocrine cells of the small intestine, known to produce C-terminally amidated peptides, suggests the existence of other amidating enzymes. (J Histochem Cytochem 41:1617–1622, 1993)

KEY WORDS: Amidation; PAM; PHM; PAL; Rat gastrointestinal tract; Gastrin; Glucagon; GLP1; Immunocytochemistry; In situ hybridization.

Introduction

The neuroendocrine system of the gastrointestinal tract consists of the neurons of the enteric plexus and a set of dispersed endocrine cells widely distributed in the epithelia of the various organs. This system is responsible for the secretion of many different regulatory peptides (Rawdon and Andrew, 1990; Polak, 1989).

Before maturation is completed, peptides undergo multiple post-translational processing steps. For some of the bioactive peptides one of these steps consists of α-amidation of the C-terminal amino acid of the precursor peptide. This α-amidation is a two-step process catalyzed by two separate enzyme activities, both derived from the peptidyl-glycine α-amidating monoxygenase (PAM) precursor, a 108 KD polypeptide (Milgram et al., 1992; Stoffers et al., 1991; Glader et al., 1990).

The NH2-terminal third of the PAM precursor contains the first enzyme, peptidylglycine α-hydroxylation monoxygenase (PHM), that catalyzes the conversion of glycine-extended peptides into peptidyl-α-hydroxyglycine intermediates. The second enzyme, peptidyl-α-hydroxyglycine α-amidating lyase (PAL), is contained in the middle third of the PAM precursor. The COOH-terminal third encodes a transmembrane domain and a hydrophilic domain that may form a cytoplasmic tail (Figure 1) (Milgram et al., 1993; Milgram et al., 1992; Ouakif et al., 1992; Eipper et al., 1991).

The presence of PAM enzymes in endocrine organs has been widely reported (e.g., Martínez et al., 1993; Braas et al., 1992; Birnbaum et al., 1989; Markosian et al., 1989). Except for the pancreatic islets (Martínez et al., 1993), expression of PAM in the diffuse endocrine system has not been studied.

The present study used rat digestive tract as a model to investigate, by immunocytochemical and in situ hybridization methods, which cells of the neuroendocrine system express PAM enzymes, and whether amidated regulatory peptides co-localize with amidating enzymes in this system.

Materials and Methods

Antisera. Two polyclonal antisera were raised against synthetic peptides of the PAM molecule as previously reported (Martínez et al., 1993). The antiserum named CC was raised to a peptide from the PHM domain of the human PAM (288–310) and antiserum PAL2 was raised to the peptide 527–546, inside the PAL region (Figure 1).

To localize endocrine cells, various polyclonal antisera raised to several