## Expression of Proadrenomedullin Derived Peptides in the Mammalian Pituitary: Co-Localization of Follicle Stimulating Hormone and Proadrenomedullin N-20 Terminal Peptide-Like Peptide in the Same Secretory Granules of the Gonadotropes

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Expression of proadrenomedullin-derived peptides in the rat, cow and human pituitary was studied by a variety of techniques. Immunocytochemical detection showed a widespread expression of adrenomedullin peptide in the adenohypophysis and the neural lobe, with low expression in the intermediate pituitary. Proadrenomedullin N-20 terminal peptide (PAMP)-immunoreactivity was also present in the anterior pituitary but showed a more marked heterogeneous distribution, with cells going from very strong to negative immunostaining. Lower levels of PAMP were found in the neural lobe. Interestingly, the distribution of adrenomedullin and PAMP immunoreactivity in the anterior pituitary did not completely overlap. In the present study, we concentrated our efforts to determine which cell type of the adenohypophysis expresses PAMP. Paraffin and semithin serial sections immunostained for PAMP and the classical pltuitary hormones revealed that a subpopulation of the gonadotropes

expresses high levels of PAM P-immunoreactive material. Ultrastructural analysis clearly showed PAMP-immunoreactivity in the follicle stimulating hormone (FSH)-containing large secretory granules of the gonadotropes, suggesting simultaneous secretion of PAMP and FSH by this cell type. Three mouse adenohypophysis-derived cell lines (AtT2O, GH3, and ccT3-1 derived from corticotropes, lacto/somatotropes and gonadotropes, respectively) were also analysed and showed expression of both proadrenomedullin-derived peptides and their mRNA. Functional studies in these three cell lines showed that neither adrenomedullin nor PAMP was able to stimulate CAMP production in our experimental conditions. Taken together, our results support that proadrenomedullin derived peptides are expressed in the pituitary in cell-specific and not overlapping patterns, that could be explained by differences in postranslational processing. Our data showing costorage of PAMP and FSH in the same secretory granules open a way by which PAMP could be involved in the control of reproductive physiology in a coordinated manner with FSH.

## Expression of Neuronal Nitric Oxide Synthase in Several Cell Types of the Rat Gastric Epithelium

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The aim of this study was to identify which cell types of the rat gastric epithelium express neuronal nitric oxide synthase (nNOS) because the results of the previous studies have been very divergent regarding this point. By the combination of immunohistochemical (IHC) and in situ hybridization (ISH) techniques, we detected expression of nNOS in chief and mucose-

cretory cells of the gastric epithelium. Moreover, some gastric endocrine cells were immunoreactive for nNOS, although they could not be distinguished in sections treated with ISH techniques. The strongest signal for all antibodies in IHC techniques was obtained when microwave (MW) heating was performed before the IHC procedure. Our results indicate that in the gastric epithelium a variety of cell types are able to produce NO. The NO produced by the different cell types (chief, mucous, and endocrine) may form a complex network of paracrine communication with an important role in gastric physiology.