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$\alpha$ -MSH during their distribution in the blood stream. It should now be established which are the physiological activities of the different forms and which signifies the post-transcriptional diacetylation process.

41. *Co-existence of TRH and Mesotocin within Nerve Fibers in Neurointermediate Lobe of the Frog Pituitary.* M. E. STOECKEL,\* C. HINDELANG,\* M. LAMACZ,† M. C. TONON,‡ AND H. VAUDRY,† \*CNRS UA 309, Univ. Louis Pasteur, 67084 Strasbourg, and †Gr. Rech. Endocr. Mol., CNRS UA 650, Fac. Sciences, Univ. Rouen, 76130 Mt-St-Aignan, France.

Both aminergic and peptidergic nerve endings are present in the neurointermediate lobe of amphibians. Immunohistochemical studies reveal that fibers containing mesotocin (MT), thyroliberin (TRH), corticoliberin (CRF), and neuropeptide Y (NPY) innervate the parenchymal cells of the frog intermediate lobe. The immunogold technique, using antisera against oxytocin (which fully cross-reacts with mesotocin), neurophysin 1, and TRH was applied either on serial sections or with a double-labeling technique consisting of immunolabeling both sides of the sections with different antibodies, revealed with gold particles differing widely in size (5 and 20 nm). In the intermediate lobe, all TRH nerve fibers appeared to react also for MT and neurophysin. The immunolabeling was related to dense-core vesicles, about 100 nm in diameter. In the neural lobe, part of mesotocinergic axones containing large neurosecretory granules (180 nm) also reacted with the TRH antibody. The TRH immunoreactive axones in the external zone of the median eminence did not react with either MT or neurophysin antibody and thus belong to a neuronal system independent of those innervating the pituitary. We used the *in vitro* perfusion technique to investigate the effects of TRH and MT on  $\alpha$ -MSH release by frog intermediate lobe cells. As reported before, TRH ( $10^{-9}$  to  $10^{-7}$  M) is a potent stimulator of  $\alpha$ -MSH secretion in amphibia. Conversely, MT ( $10^{-9}$  to  $10^{-7}$  M) had no effect on the basal secretion of  $\alpha$ -MSH. In addition, MT ( $10^{-7}$  and  $10^{-6}$  M) did not alter TRH ( $10^{-8}$  M)-induced  $\alpha$ -MSH release. Thus, the functional significance of the presence of MT in TRH-containing fibers in the intermediate lobe of the amphibians remains to be determined. (Supported by INSERM Grants 82-4019 and 84-6020.)

42. *Regulation of Synthesis and Release of Proopiomelanocortin-Related Peptides in the Pars Intermedia of Amphibians.* B. G. JENKS, B. M. L. VERBURG-VAN KEMENADE, AND A. P. VAN OVERBEEKE, Department of Animal Physiology, University of Nijmegen, Ioernooiveld, 6525 ED Nijmegen, The Netherlands.

Proopiomelanocortin (POMC) is the precursor protein for a number of peptide hormones and neuropeptides (e.g., melanophore-stimulating hormone,  $\alpha$ -MSH; adrenocorticotrophic hormone, ACTH; and the endogenous opiate  $\beta$ -endorphin). In view of the potential of POMC-producing cells to produce peptides with diverse biological activities, stringent regulatory mechanisms must presumably be functioning within these cells to determine the peptide content of the secretory signal. In amphibians the POMC cells of the pars intermedia produce and secrete melanotropins in response to the animals being placed on a black-background (background adaptation). We have examined biosynthesis, processing, and release of peptides from intermediate lobe melanotropes to study the mechanisms involved in regulating peptide composition of the secretory signal from POMC cells. These studies, conducted using the intermediate lobe of the aquatic toad, *Xenopus laevis*, have involved (1) superfusion experiments in which the secretion of immunoreactive POMC-related peptides is monitored to identify potential MSH secretagogues, and (2) pulse-chase experiments to determine possible effects that these secretagogues might have on processing of POMC within the melanotrope cell. The results show that this cell is regulated by multiple factors (both classical neurotransmitters and neuropeptides) and preliminary results are discussed which indicate that some of these factors may not only influence the rate of secretion (quantitative effect) but also influence the composition of the peptide profile of the secretory signal (qualitative effect).

43.  *$\alpha$ -MSH in the Frog Brain: Localization, Identification, and Subcellular Distribution.* C. DELBENDE,\* S. JÉGOU,\* G. PELLETIER,† M. BENYAMINA,\* D. TRANCHAND-BUNEL,\* J. GUY,† F. LÉBOULENGER,\* AND H. VAUDRY,\* \*Gr. Rech. Endocrinol. Mol. CNRS UA 650, Fac. Sciences, Univ. Rouen, 76130 Mont-St-Aignan, France; and †MRC Group Mol. Endocrinol. CHU Laval, Québec, Canada.

$\alpha$ -Melanocyte-stimulating hormone ( $\alpha$ -MSH) is a tridecapeptide amide originally isolated from the intermediate lobe of the pituitary. In addition to the hormonal action of  $\alpha$ -MSH on melanophores, intracerebroventricular administration of  $\alpha$ -MSH induces a number of behavioral effects. Thus, we have decided to investigate the possible existence of  $\alpha$ -MSH containing neurons in the brain of the frog *Rana ridibunda*. Using highly specific antibodies,  $\alpha$ -MSH immunoreactive cell bodies were observed in the ventral hypothalamic area. A rich network of positive fibers was observed in the ventral infundibular region, coursing toward the preoptic area and the