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Preface

Foreword to Special Issue: Molecular and Cellular Mechanisms of VIP, PACAP and Secretin Signaling Applied to Systems Biology

Regulatory peptide biology now faces its most interesting and challenging phase—connecting the systems biology of neuropeptide and neuropeptide receptor knock-out phenotypes in vivo, to the detailed molecular and cellular biology of neuropeptide signaling discovered in cell culture and in vitro. The potential payoff is the creation of a new, powerful, and specific pharmacology that translates into control over pathophysiological processes–neurodegeneration, chronic pain and inflammation, dysregulated proliferative signaling leading to neural and endocrine cancers, and disorders of cognition and memory–that so far have stubbornly resisted effective treatment. Neuropeptides and associated class II G-protein coupled receptors of the secretin superfamily are a particularly intriguing illustration of the task ahead. With this in mind, a Workshop entitled "Signalling Mechanisms of VIP, PACAP and Related Peptides: Contribution of Genomics, Proteomics and Bioinformatics", was organized to accompany the 7th Symposium on VIP, PACAP and Related Peptides held in Rouen, France in September 2005, with the purpose of considering how a systems biological approach to regulatory peptide signaling could best be encouraged in this field. The twelve papers that follow represent both basic research that was largely completed prior to the meeting, and review articles that reflect the discussion and focus of the Workshop itself. What they share is a growing sense that signal transduction bioinformatics, proteomics and transcriptomics can profitably be put at the service of understanding the cellular physiology of peptide signaling in vivo, especially when applied in tandem with the reverse genetics of specific neuropeptide and neuropeptide receptor knock-outs. The participants at the Workshop contributed in various ways to an exciting perspective: that this wider lens on regulatory peptide signal transduction has the potential to identify effector pathways for neuropeptide action that are both physiologically relevant, and highly specific, both requirements for effective future drug development.

Knock-outs of neuropeptides and their receptors in diverse metazoan species convincingly demonstrate that neuropeptide signaling is every bit as diversely important for systems biology–pain, circadian rhythms, neuroprotection, memory, behavior, innate and acquired immunity, neuronal proliferation and differentiation, inflammation, secretion, osmoregulation, even regulation of breathing–as originally suggested by the initial discovery of secretin's biological effects in 1901. The first paper in this issue details how knock-out of PACAP itself exacerbates pathophysiological and neurological responses to stroke, while treatment with PACAP ameliorates them. Chen et al. then use a microarray approach to identify specific target genes up-regulated in stroke that are under the control of endogenous PACAP, and those that are not. The former represent transcripts whose proteins might be directly neuroprotective such as enkephalin, or that might regulate *trans*-activate the genes encoding other neuroprotective proteins, such as Ier-3 (also called PACAP-regulated gene 1). The latter, e.g. Hsp105, may be injury effector genes that, while not controlled by endogenous PACAP, can be suppressed by exogenous PACAP and contributory to its beneficial effects in stroke. Neuroprotection elicited by PACAP is being explored also by the laboratory of Dora Reglodi, who with Akira Arimura first demonstrated the beneficial effects of exogenous PACAP in the middle cerebral artery occlusion in the rat. Here, Racz et al. demonstrate that retinal degeneration induced by monosodium glutamate is blocked by intravitreal PACAP administration through both induction of antiapoptotic, and inhibition of proapoptotic signaling, specifically involving inhibition of caspase 3 and JNK activation, and increased expression of phospho-BAD. These systems provide an excellent opportunity for examining the correlation between specific pathway activation or inhibition, and neuronal sparing, in two clinically relevant models for acute neurodegeneration with implications for chronic neurodegeneration in aging associated with dementing disease and vision loss.

Multiple connections between animal models and human clinical conditions are critical to projecting basic knowledge from the former to therapeutic application in the latter. This can be accomplished through molecular neuropathology of human cells and tissues, and basic neurochemical studies in cultured human cells. Regarding the former, Basille et al. review evidence from autoradiographic studies that there is a clear developmental shift in human cerebellum from predominantly PAC1 to mixed PAC1/VPAC receptor expression, and that adult human receptor distribution is similar to that found in rodent. This basic phenomenology is required to move forward with potential therapeutics, as well as conceptual constructs, for mechanisms of human disease. Likewise, the contribution of Muller et al.

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provides insight into the type and function of PACAP/VIP receptors present on neuroblastoma cells and more importantly, the interplay between activation of various receptor types and differentiative versus proliferative signaling in these cells. These studies are of paramount importance in projecting the potential effects of regulatory peptide therapeutics in vivo, since neuropeptide agonists will act on any receptors available to them pharmacologically, even if those receptors are not the ones that 'belong to them' anatomically and physiologically. The contribution of Dangoor et al., expands on this theme by exploring the receptor space of the VIP-preferring receptors VPAC1 and VPAC2 using branched-chain N-terminal analogs of VIP in efforts to create pharmacological agonists that have both enhanced potency, and enhanced selectivity among PACAP/VIP receptors, when administered in vivo.

The contributions of Meyer and of Lelievre et al. offer a fascinating contribution to the vexing question of exactly what regulatory role neuropeptides play in vivo in physiological versus pathophysiological processes, based on hints obtained from cell culture experimentation. Thus, Meyer explores the specific signal transduction pathways involved in neuronal progenitor cell differentiation and survival driven by PACAP-38, but then points out that, contrary to the expectations generated by such experiments, neuronal development is remarkably unperturbed in PACAP knock-out mice, and speculates that compensation by other developmental regulators may occur in these mice. Lelievre et al. supply exactly the evidence that supports such an interpretation, by demonstrating that PACAP's actions on oligodentrocyte precursors is dependent on co-signaling by RTK-activating ligands. The speculation of Meyer may be further extended to suppose that PACAP is uninvolved in *normal* development, but functions as a kind of emergency response peptide to suppress *abnormal* development, such as tumorogenesis. Waschek et al. have in fact obtained preliminary evidence, presented at the symposium to which this Workshop was attached, that the incidence of medulloblastoma is increased in mice that are heterozygous null for PACAP and for a component of the sonic hedgehog signaling pathway, suggesting that PACAP's function is indeed not to drive normal development in the nervous system, but to safeguard against abnormal proliferation driven by sonic hedgehog signaling, which is in fact required for normal proliferation underlying the development of the nervous system.

An area with a very high potential for therapeutic application in PACAP/VIP signaling is inflammation. Both VIP and PACAP have been implicated in the inflammatory response, perhaps most critically the macrophage bacterial innate response whose over-stimulation drives septic shock. Perhaps the major question in this area is whether VIP and PACAP are redundant regulators, or complementary regulators, in this process. Chorny et al. address this important issue in reviewing the receptor requirements for engagement of VIP-driven anti-inflammatory responses. Goetzl provides an additional variation on this question by putting forward the hypothesis that both plasma- and nuclear membrane-resident VIP receptors may be involved in immune response, in which case receptor binding and pharmacology of intracellular receptors adds both complexity and opportunity to regulatory peptide-based inflammatory and immune therapeutics.

PACAP is the major slow transmitter at the adrenomedullary synapse, where it has a critical function in catecholamine release and cellular plasticity during the stress response. The contributions of Guillemot et al., and Ghzili et al. address the molecular mechanisms of PACAP signaling to promote these two physiological responses. Using the secretion of EM66, a processed product of secretogranin II whose transcription is also regulated by PACAP, as a secretory marker, Guillemot et al. report that multiple protein kinases contribute to a unique sustained secretion of EM66 that may be a key component of the sustained response to stress by the adrenal medulla. On the other hand, PC12 cell differentiation is a model for the transcriptional effects of PACAP that function both during development and in driving cellular plasticity during prolonged secretion at mature synapses in vivo, both in the central and peripheral nervous systems. A second set of signaling molecules is activated by PACAP for this type of signaling, and acts on immediate early genes including Ier-3, as described by Chen et al. (vide supra), and Id3, as described by Ghzili et al. The role of Id3 is particularly interesting as uncovered in PC12 cells, since Id3 is abundantly expressed in mature chromaffin cells, and its expression may be maintained in the adrenal by chronic low-level stimulation by PACAP. CNS expression of Id3, the roles of other IEGs in PACAP action, the potential for IEG-specific pharmacological intervention and the role of microarray in identifying neuropeptide-specific signaling networks such as those underlying 'neuropeptide master regulator' actions of PACAP were subjects of discussion at the Workshop and provide fertile subjects for future investigation and development in this field.

The plethora of detailed cellular studies of neuropeptide signaling, especially by members of the class II GPCR secretin superfamily, overwhelmingly indicates that signaling can occur, mainly via cAMP and calcium, to a wide variety of intracellular third messengers such as protein kinases A, B and C, extracellular regulated/mitogen activated kinases, and ras superfamily GTPases. Contributions to an integrative understanding of the complicated and expanding field of neuropeptide signal transduction were provided by several participants who did not contribute papers to this special issue but have provided 'online' contributions, including Nancy Gough at Science's Signal Transduction Knowledge Environment, Giovanna Passafiume from Applera France highlighting interpretation of gene expression data using the pANTHER database, Alexis Lebon from Rouen describing the PreGeR-CDD database for identifying PACAP-regulated proteins, and Babru Samal discussing a plethora of web-based tools for microarray analysis and data-mining. Bioinformatics-assisted signal transduction connections provide new vistas to translate the basic findings highlighted here into exploration of downstream targets of PACAP signaling of potential clinical relevance.

Youssef Anouar
David Vaudry*
Laboratoire de Neuroendocrinologie Cellulaire et Moléculaire, INSERM U413, UA CNRS, IFRMP 23, Université de Rouen, 76821 Mont-Saint-Aignan Cedex, France
E-mail addresses: youssef.anouar@univ-rouen.fr (Y. Anouar).
david.vaudry@univ-rouen.fr (D. Vaudry).
*Corresponding author. Tel.: +33 2 35 14 67 60; fax : +33 2 35 14 69 46.

Lee E. Eiden
Section on Molecular Neuroscience, 36 Convent Drive, MSC 4090, 9000 Rockville Pike, Bethesda MD 20892-4090, USA
E-mail address: eidenl@mail.nih.gov.