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REVIEW ARTICLE

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Regulatory peptides and systems biology: A new era of translational and reverse-translational neuroendocrinology

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Abstract

Recently, there has been a resurgence in regulatory peptide science as a result of three converging trends. The first is the increasing population of the drug pipeline with peptide-based therapeutics, mainly in, but not restricted to, incretin-like molecules for treatment of metabolic disorders such as diabetes. The second is the development of genetic and optogenetic tools enabling new insights into how peptides actually function within brain and peripheral circuits to accomplish homeostatic and allostatic regulation. The third is the explosion in defined structures of the G-protein coupled receptors to which most regulatory peptides bind and exert their actions. These trends have closely wedded basic systems biology to drug discovery and development, creating a "two-way street" on which translational advances travel from basic research to the clinic, and, equally importantly, "reverse-translational" information is gathered, about the molecular, cellular and circuit-level mechanisms of action of regulatory peptides, comprising information required for the fine-tuning of drug development through testing in animal models. This review focuses on a small group of 'influential' peptides, including oxytocin, vasopressin, pituitary adenylate cyclase-activating polypeptide, ghrelin, relaxin-3 and glucagon-like peptide-1, and how basic discoveries and their application to therapeutics have intertwined over the past decade.

KEYWORDS

neuroendocrinology, regulatory peptide, therapeutics, translation

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1 | INTRODUCTION: THE SIGNIFICANCE OF 'REGULATORY'

Regulatory peptides are first messengers, released from neurones or endocrine cells, that interact with receptors on the same or immediately adjacent cells (as paracrine or autocrine factors), on synaptically-linked cells (as neurotransmitters or neuroeffectors) or on distant cells (as hormones). Regulatory peptides, along with non-peptide hormones, steroids and metabolic intermediates, including glucose and various lipids and metabolites, control the dynamics of communication between cells, contained in different organs, which defines systems physiology. The 'regulatory' aspect of regulatory peptides, however, is not contained just in their bioactivity upon release from their sites of synthesis. Rather, their availability to act in a regulatory manner at distant sites is first and foremost determined by the rates at which they are synthesised and secreted. Classic examples include: (i) pulsatile release of luteinising hormone-releasing hormone leading to the ovulatory surge; (ii) up-regulation of vasopressin synthesis and subsequent release upon changes in hydromineral status; (iii) the exquisitely fine-tuned secretion of insulin in response to glucose and a cohort of incretins, neurotransmitters and metabolites that converge on the beta cell of the pancreas through innervation and the general circulation; and (iv) the release (as neurotransmitters) from large dense-core vesicles in neurones or neuroendocrine cells only upon their appropriate stimulation by other first messengers. This can include messengers released by nerve impulses in the brain, by food in the gastrointestinal tract, or by stimuli such as pain, heat, cold, odourants and light in the sensory nervous system.

The fact that regulatory peptides are both regulated in their secretion, and act as regulators upon secretion, creates an especially intimate interaction between their study as pharmacological and physiological entities. The pharmacological effects of peptides highlight their potential therapeutic actions, although they do not always indicate when they are mimicking a physiological action. Correspondingly, physiological actions may not always translate into pharmacological ones; for example, if the dynamics of release of the endogenous peptide requires pulsatility or some other sort of episodic exposure to trigger a physiological and/or therapeutic effect.

In this review, the physiological and pharmacological actions of a few key regulatory peptides, namely vasopressin, oxytocin, glucagon-like peptide-1 (GLP)-1, pituitary adenylate cyclase-activating polypeptide (PACAP) and relaxin-3, are discussed with the intention of illuminating the broad scope of issues involved in translating peptide physiology, structural biology and pharmacology into potential therapeutic applications. The review concludes by making the argument for 'reverse translation' as a second key ingredient in achieving accelerated progress in both basic and clinical regulatory peptide science, to the mutual benefit of these two inextricably linked areas of biomedical research.

On August 10th, 2019, the Council of the International Regulatory Peptide Society (IRPS) met on the campus of the Autonomous National University of Mexico (UNAM), Mexico City, to inaugurate the IRPS as a non-profit society and to plan future meetings of RegPep, the Society's biennial meeting. A workshop held the previous day, and co-sponsored by UNAM and the IRPS, gathered together Council members to define the Society's vision for supporting and nurturing basic and translational regulatory peptide science. This vision was developed by considering some prototype regulatory peptides and approaches to their pharmacology. The workshop, entitled "Peptide Regulation in Systems Biology and Its Translational Opportunities" was designed to create a framework within which common goals of regulatory peptide physiologists, pharmacologists, biochemists, and structural biologists could be defined and future strategies developed. During the two days of activities, the IRPS also decided to seek an appropriate sponsoring journal with which to collaborate in publishing the Society's future proceedings. Accordingly, JOURNAL OF NEUROENDOCRINOLOGY was identified, the stated mission and scope of which closely matches that of the IRPS. To this end, this Perspective is intended as a manifesto for the IRPS and an announcement of its association with JOURNAL OF NEUROENDOCRINOLOGY.

2 | VASOPRESSIN AND OXYTOCIN

The regulatory peptides vasopressin and oxytocin share dual roles in mediating the physiological regulation of hydromineral balance, as well as glucose metabolism and utilisation by the brain. These homeostatic functions are in turn integrated with environmental factors and other physiological subsystems, including circadian rhythmicity, inflammation, and hormonal and autonomic feedback.[1,2] The intimate connections between (i) homeostatic regulation of salt excretion via control of kidney function by both vasopressin and oxytocin and (ii) water intake, via control of consciousness of thirst, are an exquisite example of this integration at the level of the organism.[3] In mammals, the neural circuitry for homeostatic regulation involves the control of secretion of the posterior pituitary hormones from hypothalamus into the general circulation, monitoring of blood tonicity by the circumventricular organs (CVOs) and control of seeking behaviour (thirst) by central nervous system (CNS) neurones. These homeostatic mechanisms have their earliest origins in organisms without brains, or even a CNS, although they have the capability of achieving homeostasis not by altering internal processes, but by moving to more advantageous regions of the external environment.[4] Indeed, evolution is an important touchstone in considering the potential functions of not only vasopressin and oxytocin, but also of regulatory peptides in general as a metazoan messenger class, especially when considering peptide physiology in commonly used animal models compared to human subjects.

Vasopressin and oxytocin are also involved in the prioritisation of homeostatic drives by environmental factors. This prioritisation of often competing drives is subsumed under the concept of *allostatic regulation*, and includes anticipatory thirst, as well as evaluation of salience of aversive environmental cues.[5] It is noteworthy that the control of vasopressinergic and oxytocinergic neurones and neurosecretory cells in these processes occurs in brain regions that may be either within or outside the blood-brain barrier (BBB). This should be considered in the design of drugs that affect homeostatic and allostatic mechanisms in the periphery, at the BBB and within the BBB. Environmental factors, including circadian, metabolic and gonadal status, also affect the sensitivity of vasopressin- and other peptide-containing circuits.[1,6-8] In some cases, the responsivity of peptidergic circuits as a function of inflammation or blood glucose levels can be quite marked. These factors should be taken into account when considering how drug treatments, particularly those directed at peptide receptor targets, should be tailored to individual subjects, as well as treatment regimens, in clinical trials for new neurotherapeutic drugs.

The involvement of oxytocin in specific behaviours, in addition to its well-known peripheral role(s) in reproductive functions, was highlighted in the Workshop. The role of oxytocinergic neurotransmission in the extended amygdala and hypothalamus has been explored using optogenetic and chemogenetic physiological approaches. These have revealed a dynamic role for this peptide in both effecting neurotransmission controlling real-time locomotor activity in response to environmental drives, and in the plasticity of neuronal circuits that allows the linkage of past experience to the likelihood of future responses to a given primary sensory stimulus.[9-11] Real-time changes in firing rates of peptidergic neurones are beginning to provide exceedingly clear information about what peptides do where, and when. Translating this information into increasingly sophisticated operational (including behavioural) tests for drug target engagement, and the prediction of behavioural outcomes across a broad range of subject environments, will be a goal of future translational research in the regulatory peptide domain. In that regard, recent data have indicated that oxytocin administered intranasally, and resulting in increased cerebrospinal fluid concentrations of the peptide, affects methylphenidate modulation of consummatory behaviour.[12,13] Where does oxytocin actually act to produce modulation of psychomotor stimulant effects? Determining the penetrance to brain of exogenously administered peptide will provide clues about which brain regions are actually required for target engagement for a given therapeutic application of a peptide. This type of reverse translation-oriented experimentation can provide another level of peptide specificity, through pharmacodynamics, relevant to peptide-based drug development.

3 | INCRETINS: OBESITY AND DIABETES

The worldwide obesity epidemic represents a clear and present health threat. Current interventions and treatments for obesity include lifestyle and dietary modifications, pharmacotherapy and bariatric surgery. Bariatric surgery (including gastric bypass, sleeve gastrectomy and biliopancreatic diversion) is so far the only intervention showing a long-term therapeutic effect in obesity. However, the surgical approach cannot possibly meet obesity-associated clinical/public health need in any country, developed or otherwise, across the globe. Thus, understanding the mechanisms of weight loss behind bariatric surgery will help us in translating this knowledge into less or noninvasive treatments, such as regulatory peptide-based therapies.[14] Parenthetically, the notion that patients might choose surgery, with all its attendant risks and complications, demonstrates hyperphagia, as a cause of obesity, to be a deeply rooted and highly physiological disorder that is resistant to simple voluntary restriction of caloric intake. This highlights the emerging understanding of eating as a potentially disordered behaviour with behavioural components that may involve the brain circuitry also implicated in addiction, such as to drugs of abuse.[15,16]

Along with a rise in obesity, the prevalence of type 2 diabetes (T2D) is rapidly increasing and bariatric surgery has been documented to lead to rapid remission of T2D.[17] However, curing all diabetics by bariatric surgery is impossible: bariatric surgery is life-changing and so not suitable for many individuals; it is expensive; and it remains risky. Therefore, analogous to obesity, identification of the underlying mechanism behind surgery-induced remission of T2D would open up avenues to the development of non-surgical therapies that could potentially cure T2D.

At this time, more and more regulatory peptide-based drugs for the treatment of T2D are entering the drug pipeline.[18] Clinical experience with these compounds, primarily incretin peptides, in T2D treatment has also revealed effects on weight loss, mainly through peptides acting on the GLP-1 receptor. These effects have in turn accelerated research aimed at better understanding the role(s) of peptides of both hypothalamic and ascending brain stem systems in regulating both feeding (fuel intake) and metabolism (fuel utilisation and storage). The involvement of 'incretins' at both peripheral and central loci, and in both homeostatic and allostatic function, is a scenario likely to stimulate drug discovery in the regulatory peptides in the coming decades, and in multiple therapeutic areas.

4 | PACAP: AN EMBARRASSMENT OF REGULATORY RICHES

Oxytocin and vasopressin are two regulatory peptides with a dual role in descending hormone/pituitary system function, and as ascending neurotransmitters in behavioural/allostatic function. PACAP, on the other hand, is even more widely distributed, throughout the autonomic nervous system, the hypothalamus, and the extrahypothalamic brain, providing additional challenges to integrating its physiological function and neuroanatomy.[19,20] Thus, detailed optogenetic analysis of PACAP function, compared to oxytocin and vasopressin, is less advanced. Nevertheless, work proceeding in this area with PACAP highlights the comprehensiveness of its actions in stress responses. The actions of PACAP illustrate a second major theme for regulatory peptides of the nervous system in particular. Regulatory peptides in both the brain and peripheral nervous system are almost invariably co-stored with either excitatory or inhibitory classical neurotransmitters,

including glutamate and GABA in the CNS[21,22] and acetylcholine in the periphery.[23]

A conundrum presented by some regulatory peptides. Their actions are sufficiently versatile that both antagonists and agonists for the same receptor(s) can be envisioned. This is the case for PACAP, which has been implicated in migraine (antagonist treatment at PACAP receptor[s] would be required [24,25]), depression and anxiety (antagonist treatment would be required [20,26,27]), atherosclerosis (antagonist treatment would be required [28]), tissue ischaemia (agonist treatment would be required[29-32]) and stroke (agonist treatment would be required[33-39]). A potential solution to this conundrum is the exploration of the greater specificity of action of exogenous peptides at sites of action for which either antagonism or agonism is the goal. Ligands biased for alternative signalling from a given peptide receptor is a second solution, and one that is being vigorously pursued in the development of non-addictive opiate agonists.[40] Here, it is critical to remember that any biased agonist is by definition an antagonist for the corresponding non-stimulated pathway,[41] and thus may have multiple and complex effects.

5 | RELAXIN: A PROTOTYPE 'BRAIN STEM ASCENDING' REGULATORY PEPTIDE

The relaxin-3/relaxin-3 receptor (RXFP3) system represents another example of a modulatory peptide/receptor system that is versatile in function,[42,43] although with a far more discrete, restricted anatomical distribution of peptide-positive neurones in the brain than many other peptides.[42] Indeed, a broad network of relaxin-3-containing neuronal projections arise from several small groups of neurones in the midbrain and brainstem, with the largest and best-characterised located within the nucleus incertus and others within the pontine raphe nucleus and the ventrolateral PAG.[44] Nucleus incertus relaxin-3 neurones are responsive to peripheral and sensory and stress-related inputs,[45] and project widely to RXFP3-rich areas throughout the brain, where they influence arousal,[46] hypothalamic, limbic and sensory activity,[47,48] as well as spatial memory and navigation via interactions with the septohippocampal system.[42,49,50] The pharmacology of RXFP3[51] has developed progressively over the last decade, driven by the production of RXFP3-selective chimeric, truncated, stapled and single-chain peptides,[52,53] as well as the recent report of a potent, small organic molecule agonist.[54] These important tools, which include a viral-based RXFP3 agonist delivery system,[55] along with a range of appropriate transgenic mouse lines,[56,57] will continue to assist proof-of-concept studies aiming to evaluate the involvement of RXFP3 signalling in various aspects of physiology and behaviour, and in clinical CNS disorders.[58] In turn, these studies should foster further investigations of the ability of RXFP3-related drugs to effectively treat psychiatric illnesses for which the RXFP3 signalling system emerges as a viable target.[59]

Thus, some of the lessons of GLP-1 and relaxin-3-related drug development are shared ones. In both systems, the potential for biased ligands of greater specificity is currently unclear as a result of the paucity of knowledge about the second messenger systems used by GLP-1 and relaxin receptors in different regions of the brain, although biased signalling has recently been investigated for RXFP3 and for relaxin at its cognate receptor RXFP1, in cell-based assays. Therefore this is an area of future research where investigators in both basic and applied realms can profitably contribute.

6 | PHYSIOLOGY MEETS DRUG DELIVERY: BBB, CVOS AND COMMON PEPTIDES IN THE PERIPHERY AND BRAIN

Access to the CNS is essential for the regulatory function of several circulating peptides. Indeed, some circulating regulatory peptides were demonstrated to directly cross the BBB, such as leptin, angiotensin (Ang) II, oxytocin, PACAP and ghrelin, via changes in permeability, specific transporters at the BBB, or by mechanisms as yet unknown.[12,60-62] In addition, other regulatory peptides act indirectly, rather than via penetration to CNS parenchymal receptors, to modulate neuronal function. Such indirect pathways involve the CVOs and/or vagal afferents. The CVOs are brain structures that lack a BBB and express a number of receptors for circulating signals, being able to sense the blood-borne levels of several regulatory peptides.[63,64] Similarly, vagal afferents also express a variety of receptors for circulating regulatory peptides, actively transporting receptor proteins to nerve terminals, and collecting information about local and circulating peptide levels.[65,66] Information about peripheral regulatory peptides is integrated with other CNS inputs at the CVOs and/or the nucleus tractus solitarius of the brain stem (from vagal afferents). These brain nuclei are responsible for onward transmission to those areas of the brain controlling vegetative function and behaviour, to deliver adequate neuroendocrine responses to the fluctuation of the regulatory peptides in the periphery. Besides directly crossing the BBB, and indirect action through the CVOs and vagal afferents, some peripheral regulatory peptides are also synthesised and synaptically released in the CNS, including gastrin, cholecystokinin, glucose-dependent insulinotropic polypeptide and GLP-1.[67,68]

Naturally, with multiple modes of regulation available to various peptides, whether or not CNS synthesis and synaptic release are required for the CNS action of a peptide in a given circumstance can be uncertain. Whether and when ghrelin and Ang II are actually released within the CNS, for example, has been a matter of controversy.[69,70] The use of 'sniffer cell' and other in vivo detection methods for assessment of peptide secretion in CNS is helping to answer these questions.[71] By using the sniffer cell approach, for example, Farmer et al[72] recently demonstrated the synaptic release of Ang II at the median preoptic nucleus by either electrical or optogenetic stimulation of the subfornical organ.

Peptides may act peripherally to convey information to the brain via vagal afferents; at CVOs; or directly within the brain as neurotransmitters. Therefore, it can be difficult to know in any given case (i) how the brain integrates these parallel information streams; (ii) whether therapeutic agents are acting centrally or peripherally; and (iii) whether, therefore, we can build adequate physiological models for peptide action on which to base therapeutic interventions. The

case of GLP-I is instructive here: this peptide is secreted peripherally, it is present at synapses onto neurones of the nucleus tractus solitarius of the brain stem that express both the GLP-1 receptor and GLP-1 itself, and this 'upwardly mobile' projection system acts in ventral tegmentum and other di- and telencephalic brain regions to mediate GLP-1-dependent food-specific appetitive behaviour. For these reasons, it was initially difficult, as for ghrelin, to determine whether the actions of GLP-1 are primarily peripheral, primarily central or necessarily both for a given physiological or behavioural response. It is noteworthy that this 'peptides-in-series' arrangement also exists for the presence of PACAP in both primary sensory inputs to CNS, as well as within cells upon which the latter first synapse in the brain (L. Zhang, C.R. Gerfen, V.S. Hernández, S.Z. Jiang, R.A. Barrio and L.E. Eiden, unpublished data).

7 | REGULATORY PEPTIDES, INFLAMMATION AND IMMUNE REGULATION, AND NEUROENDOCRINOLOGY

Evolution, by definition, involves the use of all the gene-encoded molecular and cellular tools available to existing organisms to maximise fitness.[73] Thus, it is not surprising that the peptides we refer to as 'neuroendocrine regulatory peptides' have additional roles outside of the realm of what we have chosen to call 'neuroendocrinology'. Examples include the roles of vasopressin and oxytocin in the thymus during self-tolerance,[74,75] roles of chromogranin peptides in autoimmune defense and disease,[76] and the role of PACAP in vascular and neural inflammation via elaboration by antigen-presenting cells including microglia and monocyte/macrophages.[77-79] In the future, we are likely to find, that these functions, appearing now to stand alone from neuroendocrinology as generally construed, are also intimately involved in neuroendocrine regulation. Systems physiology dictates that integration, rather than insulation, is the default relationship among the systems of the body, as amply illustrated by the opening example of circadian regulation of neuroendocrine susceptibility to cytokine regulation.[1,8] Perceiving regulatory peptides as first messengers that allow multicellular organisms to coordinate their activities as a single entity is an inclusive, yet 'neuroendocrine-centric', viewpoint. It is likely to be a productive one with respect to moving forward our basic understanding of mammalian physiology as an evolved enterprise, and quickly grasping how to employ new insights into neuroendocrine function into therapeutic applications.

8 | THE ROLE OF TRANSLATION AND REVERSE TRANSLATION/REVERSIBLE TRANSLATION IN REGULATORY PEPTIDE PHYSIOLOGY AND CLINICAL APPLICATION

Environmental drivers of human behaviour range from the daily appearance of the sun to the availability of food and water, interaction with other humans and domesticated mammals, and the vagaries of wind, rain and temperature that determine where human activities take place. The environment is reflected internally in the homeostatic mechanisms that promote the onset and cessation of eating, drinking, sexual behaviour and sleep, and are signalled by hunger, thirst, libido and fatigue. It is increasingly obvious to physiologists that anticipatory behaviours, referred to as allostatic regulation, are linked to homeostatic drives by regulatory peptides acting in the realms of reward and aversion, triggering behaviours of seeking and avoidance. The Workshop reflected, and the International Regulatory Peptide Society (IRPS) aims to nurture, a growing interest worldwide in understanding the properties of peptide ligand-receptor dyads in integrating virtually every aspect of mammalian physiology. Metabolic, hydromineral, sensory and social cues are linked to the prioritisation of consummatory behaviours that we recognise best when they are disrupted in disorders such as obesity, anxiety, post-traumatic stress disorder, alcoholism, drug addiction and depression, and even in some everyday events such as sickness behaviour and insomnia. The secretion of peptides from cells in the gut, adipose tissue, peripheral nervous system and brain, to sets of receptors on recipient cells, after travelling short (as neurotransmitters) or long (as hormones) distances, completes a plethora of circuits required for normal human behaviour. We are beginning to map and understand each of them, as well as the unique properties of each that offer opportunities for translation to clinical practice in a wide range of human disorders.

How do we meet the challenge of the ever-expanding roles of peptides in physiology, and the need to create ever more selective compounds for specific clinical applications? A few thoughts are worth considering. One is that, as already alluded to, peptides and their receptors have co-evolved, although with remarkable conservation of function.[80,81] This is seen quite vividly in the utilisation of peptide signalling for both homeostatic and allostatic function in the regulation of water intake and vasopressinergic signalling.[5,82] Thus, it is incumbent upon scientists who suggest potential clinical applications of a given peptidergic system for a given disease to specify, empirically, that the ligand-receptor dyad of interest is indeed embedded in the human central and/or peripheral nervous system within the same circuits identified in animal models. Often, this is not the case,[83] and human "exceptionalism" can be used to determine the best strategies for targeting a particular clinical problem in which multiple peptides may contribute. A second consideration is that peptide actions are almost invariably *combinatorial*, and this is most true when the function of interest is most primordial. Hunger, thirst and sex are existential drives for all species. The genomic niche constituted by the regulatory peptides and their receptors has clearly contributed substantially throughout the history of genetic selection (evolution), and in highly species-specific ways, to both regulating these drives homeostatically and prioritising the behaviours driven by each. The recent development of chimeric peptides that engage multiple peptidergic receptors for controlling glucose utilisation/insulin secretion provides an example of how engaging multiple players in the regulatory peptide orchestra might yield clinically impactful

results.[15] Adjudication of issues raised by clinical translation, in animal models, is a currently somewhat neglected area in regulatory peptide research. As an example, the genetic link between variation in the oestrogen-responsive element of the PACAP PAC1 receptor and post-traumatic stress disorder progression in female human subjects[84] has yet to find an analogue in a sex-specific stress response in rodents. This may be the result of a species-specific action of PACAP in humans compared to rats or mice, or a failure of reverse translation to prioritise the identification of a corresponding rodent sex-specific function for this neuropeptide in the basic physiology of stress and fear learning. However, it is likely that the issue of reverse translation will arise again and again, with important implications for both basic and translational research, in the regulatory peptide arena. The notion that translation is ever a one-way street from basic science to the clinic, as currently conceptualised, rather than an informed dialogue, is one that regulatory peptide practitioners should look forward to jettisoning.

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