COMMENTARY

Physiological signaling in the absence of amidated peptides

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Peptidergic signaling is an ancient manner of intertissue communication in multicellular organisms. Even the early eukaryote *Trichoplax*, with its limited 6-tissue repertoire, uses peptides to communicate between its tissues (1). Humans use peptidergic communication not only to transfer signals between tissues, but also to employ peptide signals in brain and peripheral nerve tracts to efficiently transfer information regarding hunger, anxiety, and many other types of physiologic states (reviewed in ref. 2). In PNAS, a study by Powers et al. (3), "Identifying roles for peptidergic signaling in mice," describes an approach to the study of peptidergic function that depends on a unique aspect of how signaling peptides are synthesized.

The manner in which signaling peptides are synthesized has remained remarkably constant over millions of years of eukaryotic evolution. Within the regulated secretory pathway (present in neurons and neuroendocrine cells), small peptides are typically excised from larger precursors by "eukaryotic subtilases" at sites marked by pairs of basic amino acids, typically Lys-Arg, followed by a series of enzymatic reactions. These reactions serve to trim, modify, and/or protect the termini of the excised peptides and are catalyzed by a variety of enzymes in addition to the subtilases. These include a specific carboxypeptidase, carboxypeptidase E, which removes terminal basic residues, and an amidating enzyme, which protects the carboxyl terminus of the trimmed peptide from degradation and often confers receptor-specific information.

This latter enzyme, peptidylglycine α-amidating monooxygenase (PAM) (Fig. 1*A*), represents a particularly fascinating molecular entity. Formed from 2 entirely different catalytic species, a monooxygenase and a lyase, in neuroendocrine cells such as neurons in the brain, this complex enzyme catalyzes a 2-step reaction that transforms a terminal glycine residue into an amide (Fig. 1*B*) (4). The importance of PAM is underscored by the fact that more than half of all known peptides are amidated, and for most of them the C-terminal amide is required for bioactivity (2, 5). In agreement with the idea that amidated peptides represent critical signaling molecules, organisms as simple as coral, *Chlamydomonas*, and *Trichoplax* use amidated peptides to accomplish complex functions (1, 6, 7).

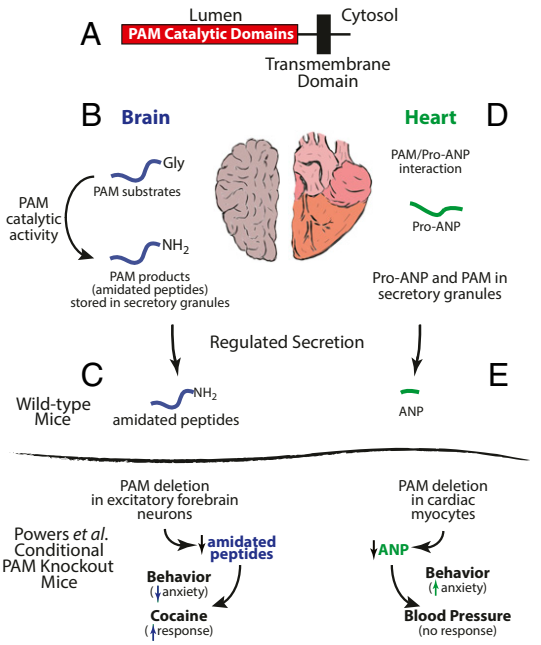

Fig. 1. Summary of the effects of PAM deletion in neurons or cardiac myocytes. Shown are (*A*) the topology of PAM, (*B*) the catalytic conversion of C-terminally glycine-extended peptides to amidated peptides by PAM in the brain, (*C*) the effects of PAM deletion in excitatory forebrain neurons on amidated peptide levels and behavior in mice, (*D*) the association of PAM and pro-ANP in the heart and the cosecretional cleavage of pro-ANP to bioactive ANP, and (*E*) the effects of PAM deletion in atrial myocytes on ANP levels and behavior. Heart and brain images courtesy of Alina Bilal (San Diego State University, San Diego, CA).

The study of neuropeptide signaling most likely reached a peak in the later part of the last century, with a total of about 100 different mammalian neuropeptides

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described by the year 2000 (reviewed in refs. 5 and 8). While the majority of peptide biosynthetic enzymes were then characterized in subsequent decades, interest in peptidergic communication within the nervous system has waned compared to interest in classical neurotransmitter systems (glutamate, dopamine, norepinephrine, and acetylcholine), largely due to the emergence of powerful new optogenetic and chemogenetic tools (as well as voltammetry) to study brain pathways containing the latter. Far fewer tools are presently available to study peptidergic pathways. While genetic models of specific peptide deficiency have been developed, these are largely restricted to whole-body gene deletion; and changes in peptidergic transmission cannot presently be quantified at relevant spatial and temporal resolutions. Thus, it has been difficult to assess the precise contribution of peptidergic signaling in specific cell types.

The paper by Powers et al. (3) begins to address the lack of genetic tools to study neuropeptide function. In an effort to restrict the analysis of amidated peptidergic function to specific tissues and cell types, Powers et al. (3) constructed a floxed PAM mouse and bred this mouse with 2 different site-specific Cre recombinase-expressing driver lines to examine the contribution of peptidergic transmission to function in several specific cell types. This is a critical technical advancement because of the widespread importance and function of amidated neuropeptides, which is underscored by the fact that global PAM deletion in mice is embryonic lethal (9).

In the first mouse line described by Powers et al. (3), PAM was conditionally deleted from excitatory forebrain neurons, presumably altering the balance of excitatory and inhibitory signaling in circuits involving these neurons. While these mice exhibited no notable differences in weight or motor activity from wild-type mice, they were significantly less anxious than wild-type mice in an elevated 0 maze test (Fig. 1*C*), supporting Powers et al.'s (3) speculation that amidated peptides produced in excitatory glutamatergic neurons play functionally important roles. Another interesting behavioral feature of these mice was observed when testing responses to cocaine, during which they exhibited a significantly enhanced locomotor responsiveness. While these results, perhaps indicating altered amygdala peptide signaling (10), cannot be ascribed to a specific amidated neuropeptide, they provide the basis for further exploration using finer anatomic and chemical tools and may yield additional drug targets to combat addiction.

In the second mouse line, Powers et al. (3) conditionally deleted PAM from cardiac myocytes; they did this because they had previously found that PAM is expressed at the highest levels in atrial myocytes (11, 12), which are well known for making the natriuretic depressor hormone, atrial natriuretic factor (ANP) (13). It was previously found by others that ANP is stored in large quantities in atrial myocytes as an inactive prohormone, pro-ANP, and that it is proteolytically processed to its active form, ANP, at the moment of secretion (Fig. 1*D*) (14). While this unusual peptide prohormone processing event is well understood, the reasons for cosecretional proteolytic processing are not known. The finding of large quantities of PAM in atrial myocytes, where it is colocalized with pro-ANP (15), is particularly intriguing because ANP is not amidated and none of the other major peptides made by atrial myocytes are amidated. These findings suggest that PAM, which is a secretory granule luminal protein in most neuroendocrine cells, might serve additional functions in the heart, where it resides as a transmembrane protein in the Golgi complex and in atrial secretory granules.

In support of additional functions for PAM in the heart are studies showing that pro-ANP, while itself not a transmembrane protein, is tightly associated with atrial secretory granule membranes, probably by virtue of its binding to the intraluminal portion of membrane PAM (16). This interaction implies that PAM and pro-ANP might collaborate to exert as-yet undiscovered functions perhaps unrelated to peptide amidation. Consistent with this are studies showing that disrupting the association of PAM and pro-ANP in atrial myocytes, which requires mutating only 2 amino acids in pro-ANP, impairs atrial secretory granule biogenesis, as well as ANP secretion (17–19). These results suggest that, in addition to its known role in peptide amidation, PAM in atrial myocytes may function in concert with pro-ANP to facilitate the biogenesis of secretory granules. While the authors did not examine the effect of PAM deletion on atrial myocyte secretory granule number, they did find that deleting PAM specifically in cardiac myocytes decreased atrial levels of pro-ANP and circulating levels of ANP. Coupled with the mutation studies showing that disruption of the PAM/pro-ANP interaction decreases atrial secretory granule number, this finding underscores an essential role for PAM in the heart and provides a potential explanation for why ANP is stored in the heart as a precursor.

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ANP is known to decrease plasma sodium and thus works to maintain normal blood pressure. However, Powers et al. (3) find that while PAM deletion in cardiac myocytes decreases ANP levels, it does not result in the predicted increases in blood pressure in mice subjected to a high-salt diet. This result is surprising, since global knockout of ANP in mice results in hypertension, even in mice on a normal salt diet (20). Another, perhaps more surprising finding is that deletion of PAM in cardiac myocytes results in behavioral effects, including an increase in anxiety-like behavior (Fig. 1*E*). Although the connection between PAM deletion in cardiac myocytes and brain function that probably underlies this phenotype is not yet understood, it could be that atrial ANP, or some other atrial factor(s) impacted by PAM deletion, can signal back to the CNS in exciting, yet-to-be described ways.

While the Powers et al. (3) paper provides an important step forward in providing an approach to analyzing peptide function, complicating matters is the possibility that the loss of PAM results in other physiological effects distinct from and additional to decreased peptide amidation. In fact, this possibility is underscored by the abovementioned findings in PAM-deleted atrial myocytes. Recent studies have shown that PAM expression also plays a role in intracellular communication in endocrine cells, with its carboxyl-terminal tail shuttling to the nucleus to convey information regarding secretory granule content (21). Indeed, the full range of functions for PAM may not yet be known, as PAM is found in cilia and seems to play an evolutionarily conserved role in ciliogenesis (22). Parsing out these noncanonical roles for PAM from its direct role in peptide amidation may prove to be complex.

In summary, with the development of the PAM floxed mouse, the Powers et al. (3) paper represents the start of an additional toolkit for exploring peptidergic signaling in a cell-specific manner. Others will undoubtedly build on this model to refine the contributions of specific amidated peptides; for example, one could envision selective restoration of NPY expression using AAV-proNPY injection into specific brain nuclei containing proNPY-expressing cell bodies. Spatially selective peptide loss could

potentially be achieved by viral injection of Cre-expressing AAVs, again with the caveat that cell bodies rather than terminal fields must be targeted. The ability to accurately assess the physiological contributions of peptide signaling to specific circuits, known to occur in concert with classical neurotransmitter signaling, will hopefully usher in a renaissance of the study of neuropeptides, which is sure to provide a much richer mechanistic picture of signaling overall.

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