An Intracellular Renin-Angiotensin System in Neurons: Fact, Hypothesis, or Fantasy

The renin-angiotensin system in the brain acts to regulate a number of physiological processes. Evidence suggests that angiotensin peptides may act as neurotransmitters, although their biosynthetic pathways are poorly understood. We review evidence for neuronal production of angiotensin peptides and hypothesize that angiotensin may be synthesized intracellularly in neurons.

In 1961, Bickerton and Buckley (3) first demonstrated that Ang-II acts directly within the central nervous system to increase blood pressure. It was further demonstrated that central injection of purified Ang-II (specifically around the hypothalamus) resulted in a robust drinking response (7). These reports firmly established a role for central Ang-II in the regulation of blood pressure and hydromineral balance and implied that Ang-II-sensitive receptors are present within discrete regions of the brain. Other studies documented the presence of renin within the brain, thereby providing evidence for endogenous angiotensin production within the central nervous system (9, 12). Together, these early studies established both the production and distribution of Ang-II within the central nervous system.

With these data now in hand, current brain-RAS researchers are left with a discrete set of questions: Since the RAS ultimately functions by stimulating cell surface receptors, which induce intracellular signaling mechanisms, where do these signals originate, where do they act, and how are they transduced? In other words, what portions of the brain (both regional and cell specific) synthesize angiotensin peptides, and which portions express receptors for these peptides? What are the functions of the RAS within the brain in terms of behavioral and physiological regulation? Are angiotensin peptides utilized by neurons as neurotransmitters and/or neuromodulators?

Regulation of peripheral organ system function by the brain is mediated through either direct synaptic contact (such as the innervation of skeletal muscle, cardiac muscle, and various glands) or neurohormonal signaling (such as vasopressin or ACTH release into the circulation). In both cases, regulation of anatomically and chemically distinct neuronal populations mediates these functions. To understand exactly how the central RAS can modulate physiological function, then, it should be our first goal to define the molecular and cellular localization and actions of the RAS within the brain. Determining, for example, whether angiotensin peptides are used as neurotransmitters (versus neuromodulators) is key to our understanding of how the central RAS can control neuronal function.

In their textbook, Cooper, Bloom, and Roth (4) have described a five-point definition for what constitutes a neurotransmitter: 1) the substance must be synthesized in, and released from, the presynaptic neuron; 2) this substance must be released from nerve terminals (into synapses); 3) the substance must cause effects post-synaptic neuron must be receptor-mediated and dose-dependent; and 4) an active degradation or reuptake mechanism must exist. Several excellent reviews (most notably Ref. 8) have outlined how Ang-II fulfills many parts of this classic definition, with the notable exception of the first, neuronal synthesis. We will focus on the evidence for neuronal synthesis of Ang-II, the first criteria required to define Ang-II as a neurotransmitter. Furthermore, we examine how the discovery of new components of the RAS, namely an intracellular form of renin type 1 (PPI) higher in the old-type litter.
the renin enzyme and the (pro)renin receptor (P[RIIR]), may help provide mechanisms to achieve neuronal synthesis of Ang-II.

Angiotensin synthesis in the central nervous system

All of the angiotensin peptides are produced by sequential enzymatic cleavage of angiotensinogen, and alternate processing pathways result in the irreversible formation of subsequent peptides (FIGURE 1). Thus the minimum requirement to generate all angiotensin peptides is the synthesis of angiotensinogen. The majority of angiotensinogen synthesized within the brain is produced by astrocytes where it is constitutively secreted into the interstitial space and cerebrospinal fluid (5, 51). Giall angiotensinogen has been presumed to be the substrate through which exogenous renin can cause pressure and diisogenic responses, when the enzyme is administered directly into the brain ventricles (15). Indeed, the enzyme is administered directly into the brain, thus leading some to hypothesize that angiotensin peptides may be formed through renin-independent mechanisms. Arguing against a renin-independent mechanism are genetic data using transgenic mouse and rat models that take advantage of the strict species specificity of the enzymatic reaction between human renin and human angiotensinogen (13, 26). In nearly all of those species, animals expressing a single transgenic renin or angiotensinogen (or both) have an immediate rise in blood pressure. On the other hand, animals expressing renin in glial cells or glial angiotensinogen in neurons, is there evidence for neuronal Ang-II staining? Ang-II-stained magnocellular and parvocellular neuronal cell bodies are abundant in the PVN and supraoptic nuclei (SON), and Ang II has also been reported in other hypothalamic nuclei, the enzymatic cleavage of angiotensinogen has been presumed to be synaptic vesicles (35). Furthermore, Ang II has been co-localized with aminobutyric acid in nerve terminals near synapses, and some Ang II is evident in secretory vesicles, which may be synaptic vesicles (35).

Given the presence of the angiotensin precursor angiotensinogen and the effector Ang II in neurons, where is the renin? It is commonly accepted that the first and rate-limiting step in the production of all subsequent angiotensin peptides is the synthesis of angiotensinogen by renin. Therefore, by definition, a local RAS can only exist in the brain if renin is also present or can gain access. Renin-like enzymatic activity was first demonstrated in dog and rat brain homogenates (9, 12). Further studies demonstrated that renin protein and mRNA were present in astrocytes and neurons in rat, mouse, and human (6, 11, 14, 46). The localization of renin to particular structures and cell types within the brain has proven difficult, however, since its levels within the brain are very low. No doubt this has been a major technical hurdle toward understanding the biosynthesis of Ang II in the brain, thus leading some to hypothesize that angiotensin peptides may be formed through renin-independent mechanisms. Arguing against a renin-independent mechanism are genetic data using transgenic mouse and rat models that take advantage of the strict species specificity of the enzymatic reaction between human renin and human angiotensinogen (13, 26). In nearly all
of angiotensinogen expressing cells within the SFO, PVN, and RVLM (17). Finally, we have demonstrated similar activity. In interpreting these findings, one must not only consider that the use of transgenic rats and mice either overexpressing or ablating the synthesis of RAS components in the brain has resulted in a wealth of new detailed information on the role of the RAS in regulating blood pressure and hydromineral balance."
thus may result in the ablation of transgene expression in neurons that project to the injection site (44). Consequently, the reduced pressor response to human renin in single transgenic mice and the reduced water intake in the double transgenic mice may be due to loss of human angiotensinogen in the SFO and also in neurons projecting to the SFO. Nevertheless, both studies collectively highlight the importance of local angiotensin production within the brain.

**Novel players in the RAS: intracellular renin**

The classical renin mRNA transcript contains nine exons in rodents (10 exons in humans) and has a very short 5' untranslated region (UTR) (FIGURE 2). Translation begins at the first ATG codon in exon 1. In juxtaglomerular cells of the kidney, the primary product of renin mRNA translation is preprorenin. The “pre” represents the signal peptide that directs the protein to enter the endoplasmic reticulum with subsequent incorporation of the polypeptide into the secretory pathway. After cleavage of the signal peptide, the remaining protein (prorenin) is sorted into either the constitutive secretory pathway (and is passed directly out of the cell without further processing) or the regulated pathway where the protein is glycosylated, packaged into secretory granules, and the prosegment removed. The prorenin converting enzyme, which processes inactive prorenin to active renin, remains unclear. It is also unclear whether this pathway is shared in other renin-expressing cell types and tissues, including the brain. We and Lee-Kirsh et al. reported that the structure of renin mRNA in the brain is different from that transcribed in the kidney (21, 42). Instead of transcription beginning at the classical renin promoter and containing exon 1 (now termed exon 1a), the transcripts in the brain start at a unique transcription start site and contain a new first exon (termed exon 1b, encoding renin-b). Renin-b mRNA initiated at exon 1b splices directly to exon 2, and thus the remainder of the mRNA is out of the brain without further splicing (FIGURE 2). Since there is no exon 1a, translation cannot initiate from the “normal” ATG present in exon 1a, and, importantly, there are no ATG sequences in exon 1b. Thus translation must initiate at the next “in框架” ATG, which lies in exon 2. Interestingly, this ATG is extremely well conserved throughout evolution, more so than the surrounding sequences in the prosegment. Translation from the exon 1b-ATG would result in the production of a protein devoid of the signal peptide and the first third of the prosegment. Biochemical studies revealed that renin-b is active, suggesting that the remaining two-thirds of the prosegment is insufficient to inhibit renin activity (21). It is therefore hypothesized that translation of the renin-b transcript produces an intracellular form of active renin. Interestingly, mutations in the renin prosegment caused both an increase in enzymatic activity of prorenin but also decreased secretion in transfected CHO cells (25). This led the authors to conclude that the prosegment was required for efficient expression of renin. Along these lines, it is notable that renin levels in the brain are extremely low.

We examined the hypothesis that expression of the intracellular form of renin in the brain would result in increased arterial pressure by comparing two double transgenic mouse models, each expressing human angiotensinogen and glial-specific expression of either intracellular renin or secreted renin. Both models exhibited an equivalent increase in arterial pressure dependent that was AT1 receptor driven, providing evidence for a physiological function of intracellular renin in the brain (19).
Recent studies in non-neural cell types in culture suggest that intracellular levels of angiotensinogen, renin, and Ang II can be modulated under certain conditions (41). Consequently, this begs the question of whether the identification of this novel brain-specific transcript encoding an intracellular active renin is the missing link in defining Ang II as a neurotransmitter. Clearly, there are many unresolved questions derived from this observation (FIGURE 3). Are renin-b mRNA and protein localized in neurons in regions of the brain controlling cardiovascular function? Is renin-b sorted to an intracellular compartment where it could interact with angiotensinogen, ACE, nephrilysin, ACE2, or aminopeptidases to form Ang II, Ang-(1–7), or other smaller angiotensin peptides? Does this intracellular processing of angiotensinogen, if it occurs, result in the production of angiotensin peptides that are sorted directly to the neurosecretory apparatus?

We have begun to take steps toward answering these questions. For example, we have recently determined that Neuro-2a neuroblastoma cells express renin-b mRNA, and we are screening other neuronal cell types for expression of this renin isoform. We have also embarked on an ambitious project using gene targeting in C57BL/6 embryonic stem cells to take advantage of the structural differences in the gene encoding the renin-a and renin-b mRNAs to generate separate "null" and "flox" alleles of the renin-a and renin-b mRNAs derived from the mouse Ren-1c gene. Some of these mice have already been obtained, and their analysis should provide novel tools and insights that will allow us to dissect the relative importance of these two renin isoforms in the brain.

**Novel players in the RAS: the (pro)renin receptor**

In addition to the direct synthesis of intracellular active renin, the discovery of a receptor for (pro)renin provides a possible mechanism by which neurons could either concentrate or potentially sequester extraneuronal sources of renin. The (pro)renin receptor, first identified by Nguyen et al. (32), binds both renin and prorenin, nonenzymatically activates prorenin to increase its angiotensinogen-cleaving catalytic efficiency, and activates intracellular signaling...
EMERGING TOPICS

pathways. Of particular interest is the observation that this receptor is expressed at high levels within the brain. Is it possible that, through binding to neuronal (pro)renin receptors, local renin levels may be substantially increased near (or dependent of the original source of the prorenin or neurons or glial cells. As of now, there are few data implicating a role for this receptor in the brain. As above, many questions would need resolution. Are (pro)renin receptors localized on neurons in close proximity to AT1 receptors and are they present in high levels or neuronal AT1 receptors and concentrate renin directly in the proximal source of Ang II derived from angiotensinogen released from either neurons or glial cells. Of particular interest is the observation that this receptor is

References

31. Mungall BA, Shinkel TA, Sermia C. Signalling across the blood brain barrier by 10.220.32.247 on August 20, 2017 http://physiologyonline.physiology.org/ Downloaded from