During the past 3 to 4 decades, there has been debate on the presence as well as the role of local tissue renin–angiotensin systems (RAS) in cardiovascular physiology and pathophysiology. Although it is now accepted that all components of the RAS are present in a variety of extrarenal tissues and their regulation may be independent of the circulating hormonal system, the factors contributing to this regulation are still not well understood. Also apparent is that our understanding of the relationships among various cell types expressing individual RAS components and production of specific angiotensin peptides in each tissue is still lacking. This is particularly true of the brain RAS, where even after 20 years the question of whether there are angiotensinergic versus reninergic neurons or a complete functioning RAS in cerebrospinal fluid (CSF) or extracellular fluid or glia remains in question.

Ever since early reports provided biochemical evidence of RAS components in brain, controversy regarding their cellular localization, independence from the circulating system, and authenticity of the proteins and peptides persists. It is well accepted that angiotensinogen is present in CSF/interstitial fluid and localization via immunocytochemistry and in situ hybridization histochemistry reveals that production of the precursor protein is primarily in glia, but also in neurons, within key cardiovascular nuclei. Lingering questions remain concerning local expression of authentic renin in tissues, especially given that prorenin or active renin can be seques-
tered from the circulation and other enzymes can exhibit similar proteolytic profiles under certain conditions. However, there is unequivocal evidence of discrete cells within the pituitary, choroid plexus, medulla oblongata, and hypothalamus that are positive for renin immunoreactivity colocalizing mainly with neurons, but in the medulla oblongata and subfornical organ, in glial elements as well. Evidence of renin mRNA in brain tissue provides a mechanism for local synthesis of the protein. Using green fluorescent protein driven by the renin promoter, studies revealed predominant but not exclusive presence of renin mRNA in neurons. All enzymes required for subsequent processing of angiotensin I into active peptides, including converting enzyme (ACE) for angiotensin II, ACE2 and neprilysin for angiotensin-(1-7), and aminopeptidases for angiotensins III and IV, are present on the basis of immunocytochemical or molecular approaches. The predominant localization of these secondary enzymes on plasma membranes is interpreted as evidence for extracellular formation of the final bioactive peptide products.

Finally, evidence of receptors defined by molecular, functional, or binding methods for angiotensins II, III, IV, and (1-7) complete the brain RAS. The receptors are widely distributed throughout cardiovascular and neuroendocrine control centers as well as in areas of the brain more typically considered as part of motor and sensory processing and memory and affective behaviors. However, this is consistent with the widespread actions of the angiotensin peptides in brain.

Molecular approaches developed in the past 15 to 20 years yielded substantial progress in establishing firmly the presence and, to some extent, the cellular localization of processing enzymes and precursor substrate of the brain RAS. A significant insight with respect to the importance of renin, specifically in blood pressure control and hypertension, arose when intraventricular administration of renin mRNA antisense oligodeoxynucleotides lowered blood pressure in spontaneously hypertensive rats (SHRs). These findings are consistent with many other studies using a variety of RAS inhibitors (molecular or pharmacological) administered into brain ventricles or specific brain nuclei to reveal major contributions of brain RAS to hypertension in SHRs. Because the SHR is a genetic form of hypertension exhibiting overactivity of one or more components of the brain RAS, amelioration of hypertension by blockade of brain renin clearly supports the concept that upregulation of the brain RAS participates in hypertension. The chronic phase of renal hypertension is another example where an activated central RAS plays a role in hypertension. The powerful influence of brain angiotensin II on cardiovascular and renal function was elegantly demonstrated in studies where an angiotensin II–generating construct was inserted behind a glial fibrillary acidic protein promoter in angiotensinogen deficient mice. This replacement of angiotensin II rescued the renal defects characteristic of systemic deficiency of angiotensinogen.

Despite the use of molecular tools to demonstrate the presence and function of the brain RAS, we do not have a clear concept of the configuration of the brain RAS for processing of angiotensinogen by renin or any other potential candidate proteolytic enzyme for that matter. Processing may occur in the extracellular spaces, including CSF, as a result of transfer of precursor enzymes and substrates between cells or within a single cell. In part, this question arises because of the limited localization in brain tissue of cells expressing renin.
and the predominance of renin in neurons and angiotensinogen in glia. In addition, although localization of peptides of interest would be desirable to address this issue, these can only be detected by immunocytochemical methods and the processing necessary for tissue preparation may in itself "wash out" or lead to degradation of extracellular peptides. Even with evidence of angiotensin peptides in neurons or glia, their origin remains controversial, as their presence may result from synthesis within the cells or as a consequence of uptake by receptor-mediated endocytosis.

Efforts by Bader, Ganten and colleagues, and Sigmund and colleagues during recent years have been directed toward unraveling the mystery of the configuration of the brain RAS and whether glial or neuronal elements participate independently or in concert in generation of active peptides. In their most recent article in this issue of Hypertension, Lavoie et al address the question of whether intracellular or secreted renin overexpression contributes to similar or different cardiovascular phenotypes. The rationale for their study arose in part from observations that splice variants coding for a nonsecreted form of renin, representing the major form of mRNA in brain of several species, might imply an intracellular localization of the protein. In addition, a previous report suggested both colocalization of renin and angiotensinogen to the same cell and expression of the 2 proteins in adjacent cells using fluorescent markers for the respective promoters. Thus, the issue of whether one cell is capable of producing active peptide remained controversial.

The present article demonstrates that overexpression of an intracellular human renin when coexpressed in glial cells also expressing human angiotensinogen can generate high blood pressure dependent on angiotensin type 1 receptor activation. Although a limitation of the study is that expression of renin protein may alter expression of other RAS components within that cell, creating an artificial situation, this would still be evidence that glial cells expressing renin and angiotensinogen are capable of generating angiotensin peptides. Unfortunately, even in this study, it is not known whether enzymatic steps between formation of angiotensin I and other active peptides occur within the same glial cell, adjacent glial or neuronal cells, or extracellularly by membrane-bound enzymes located on glia or neurons.

Animals with intracellular renin overexpression differed in the magnitude of the elevation in drinking behavior, despite similar increases in blood pressure. Since there was no information on regional distribution of expression of the human renin, different patterns for expression of intracellular versus secreted human renin may be responsible. Additionally, with intracellular human renin overexpression there was an increase in circulating mouse renin. This is an intriguing observation since it implies that activation of the glial RAS initiates a positive feedback on the peripheral system presumably through activation of renal sympathetic nerve activity and kidney renin release, despite the elevation in blood pressure. The expected accompanying increase in circulating angiotensin II could actually be a contributor to the increase in blood pressure and drinking as the peptide may act at sites accessible by intraventricular administration of losartan.

An important issue raised by the Lavoie et al article is relevant to glial versus neuronal origins of components of the brain RAS. Whether there are distinct actions of angiotensin peptides derived from either source remains an interesting area of active investigation. The data from Lavoie et al are interpreted that peptides derived from components of glial origin promote hypertension and polydipsia. Indeed, previous work shows that glial overexpression of human angiotensinogen and human renin in a double transgenic mouse leads to impairment in baroreflex sensitivity and an increase in resting blood pressure. In contrast, overexpression of components in neuronal elements alters the baroreceptor set point rather than sensitivity in addition to elevating blood pressure. However, these studies involve an increase in human renin and human angiotensinogen in all astrocytic glial cells or all neurons, making it difficult to translate findings into a setting of expression of components behind their normal promoters for localization within the proper brain areas.

Another approach to address the importance of the origin of the precursor protein is the transgenic rat (ASrAogen) with targeted disruption of the glial source of angiotensinogen by angiotensinogen antisense behind a glial fibrillary acidic protein promoter. The important insights into consequences of loss of glial-derived substrate from cells normally responsible for its production include an enhanced sensitivity of the baro- and chemoreflexes in these animals relative to rats with overexpression of brain renin, along with lower resting arterial pressure. The findings are very consistent with the above mentioned data showing impairment in reflex sensitivity present in mice with overexpression of glial, but not neuronal, angiotensinogen and renin. The ASrAogen rats have lifelong lower blood pressure and impaired renin release from the kidney in response to stress. As further evidence of the important role of the brain RAS in manifestations of systemic cardiovascular pathophysiology, these animals have improved indices of cardiovascular and metabolic functions as they age and are protected from certain aspects of cardiac damage in response to both coronary artery ligation and angiotensin II–infusion hypertension. While brain tissue levels of angiotensin I and II are reported to be modestly lower in ASrAogen rats, angiotensin II and (1-7) in neuronal pathways of the paraventricular nucleus and medullary autonomic centers are comparable between Sprague-Dawley and ASrAogen rats. The source of neuronal peptides would appear to be other than glial-derived angiotensinogen; however, whether they represent uptake from blood or CSF via transport mechanisms involving receptor internalization or neuronal synthesis, either intracellular or a combination of intracellular and extracellular events, remains unknown. Regardless of the details of processing, transgenic animals reveal that angiotensin peptides of glial origin may represent neuromodulators, whereas the neuronal-derived peptides contribute to transmitter-like effects of the brain RAS.

Further complicating our understanding of the role of the intrinsic brain RAS in overall regulation of the cardiovascular system is the difficulty in separating effects of blood-borne angiotensins acting at receptors localized in brain circumventricular sites. Certainly, in the above examples of angiotensin II–induced hypertension in ASrAogen and responses of these
animals to behavioral stress, an interaction of circulating and intrinsic brain RAS is likely. Moreover, the effects of RAS blockade systemically cannot be interpreted without considering actions of these agents at sites both outside and within the blood-brain barrier.

Thus, despite major strides concerning establishment of all components of RAS within neuronal, glial, and epithelial (choroid, ependyma) elements and recent proof that each source of peptides plays an important yet distinct role in cardiovascular and neuroendocrine balance, we lack the critical understanding of how the system is configured during either normal participation of the brain RAS in cardiovascular and neuroendocrine functions or when the RAS is activated under pathophysiological conditions. Future efforts mandate transgenic animals with targeted cell and tissue specific overexpression and, most importantly, underexpression of RAS components, especially the processing enzymes specific for each peptide, behind native promoters. Subsequent challenges to activate or suppress the brain RAS could then be used to fully understand the importance of specific potential sources of RAS enzymes and substrates involved in normal physiology and pathophysiology.

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References

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