Is There a Brain Isorenin-Angiotension System?

The following comments are offered in reply to the review by Ian A. Reid in Circulation Research 41: 147-153, 1977.

Brain Isorenin

The optimum of enzyme activity is quoted to be at pH 4.5 to 5.0 with no measurable activity at pH 7.4. Our results agree with Reid on the optimum of enzyme activity. Whether there is product formation at the given proton concentrations or not is a merely quantitative question, however, depending on how much enzyme is used in an assay. We do find angiotensin I formation at neutral pH given sufficient quantities of enzyme and angiotensinogen. Other authors have reported an optimum of rat brain renin activity at pH 7.0 to 7.5; extrarenal isorenin from the submaxillary gland has an optimum at pH 8.0. This discrepancy of results needs to be mentioned and may be of biological and methodological importance. Reid uses the low pH optimum of enzyme activity as an argument against its biological role. If this is correct, many acid proteases would have no biological activity, but enzyme characteristics are dependent on the in vitro incubation conditions. Kidney renin and brain isorenin exhibit different pH optima with different angiotensinogens. This is also known for other enzymes. Furthermore, the in vitro characterisation, though necessary, has little consequence for its function in vivo. An enzyme that catalyses the cleavage of a specific product (angiotensin I) from a high molecular weight precursor (angiotensinogen) and does not destroy the product (no angiotensinase activity) is a good candidate for angiotensin I formation in vivo. It is quite conceivable that the enzyme-prohormone reaction takes place in intracellular compartments; here any pH can be realistic. The fact that brain isorenin (like kidney renin) is located in lysosomal-like granules is in favour of this hypothesis. A well-studied system of intragranular hormone synthesis is the formation of insulin from proinsulin by trypsine-like enzymes.

Reid states that the human brain isorenin has no activity against human angiotensinogen. From the paper of Daul et al., it is evident that there is some activity, and that the main problem of studying kinetics of isorenin with human angiotensinogen is caused by high substrate blanks due to contamination of the plasma substrate with plasma renin. If plasma angiotensinogen is obtained from nephrectomized animals, this contamination can be avoided, and it has been found that homologous plasma angiotensinogen reacts well with brain isorenin in various species. We also found activity of purified human brain enzyme with purified human angiotensinogen. Furthermore, Hoffman et al. have reported that homologous angiotensinogen from the rat injected into the brain does induce drinking in nephrectomized rats. This effect is mediated by angiotensin generated through the local action of isorenin on the exogenous substrate and can be suppressed by inhibition of brain-converting enzyme and blockade of angiotensin receptors. There is thus evidence that brain isorenin reacts with homologous substrate in vivo and in vitro.

Brain Angiotensin

Reid states that the apparent angiotensin immunoreactivity in brain extracts may be an artifact caused by angiotensinase activity. It is one of the prerequisites with all radioimmunoassay techniques to work in systems which do not contain contaminants that could destroy the labeled antigen. This can be controlled for easily by inactivation of enzymes (e.g., boiling, acid treatment) and it would be recognized in the laboratory, if serial dilutions of the sample do not fit the standard curve. It simply cannot be assumed that all groups worked with such inappropriate radioimmunoassays. In all studies where extraction was done by acid or alcohol or where chromatographic purification of angiotensin was included in the procedure, there will be no angiotensinase activity present and such errors are excluded a priori. In these studies a peptide has been extracted from brain that corresponds to angiotensin by all classical criteria. It may well be that cross-reactivity of the antibody with angiotensinogen or other phenomena such as protein binding may provide an answer to conflicting results. Results obtained by extraction with 6 M urea to dissociate protein complexes are indicative of an angiotensin-protein complex in brain.

Angiotensin Blockade in Vivo

It is well recognized that the spontaneously hypertensive rats used in different laboratories are not identical with respect to their hormonal status and with respect to the activity of the renin-angiotensin system or brain isorenin-angiotensin system, even though most of them are originally derived from the Wistar Kyoto strain. Certainly no completely homogenous effect of central angiotensin blockade can be expected in these rats in different laboratories where testing techniques and rat diets also may vary widely. For example, we have reported that one such strain of spontaneously hypertensive rats shows no response or a blood pressure increase upon central application of saralasin and the same occurs in mineralocorticoid-induced hypertension with positive salt balance. In spontaneously hypertensive rats of the New Zealand strain and in the hypertensive rats of the stroke-prone Wistar Kyoto strain, we have consistently found blood pressure decreases following angiotensin blockade. Indeed, the reduction of blood pressure in spontaneously hypertensive rats by angiotensin blockade is one of the strongest arguments in favour of a biological role of the brain isorenin-angiotensin system and has been confirmed by several groups. The decrease of blood pressure in rats with the brain ventricular system perfused with artificial cerebrospinal fluid (CSF) does not question the specificity of the hypotensive effect observed with saralasin but
rather supports it because the angiotensin II will be washed away from the periventricular angiotensin receptor sites. Furthermore, it has been reported that the blood pressure-decreasing effect of saralasin was still present during the perfusion with artificial CSF, but was attenuated to the same extent as the blood pressure had decreased by the washout. This is exactly what would be expected when angiotensin is indeed washed out by the perfusion with artificial CSF. There is now evidence that the saralasin effect persists in nephrectomized spontaneously hypertensive rats, another argument supporting the role of brain angiotensin in the maintenance of high arterial blood pressure in these animals. Angiotensin receptors in brain are mediating these effects. They are described in various regions of the brain in different species. Brain angiotensin receptors with high affinity, specificity, and reversibility of binding are well characterized, but not discussed by Reid.

Most of the findings concerning the brain isorenin-angiotensin system have been confirmed by several groups. It would be surprising and indeed a joke of nature if its components were present but the system would have no biological role in brain. It may well be, however, that functions totally different from those traditionally linked with the renin-angiotensin system may be discovered for brain angiotensin. The mere presence of opiate receptors has led to the discovery of opiate-like peptides in brain. The renin-angiotensin system is the only peptide-generating system so far in brain, for which all components (prohormones and enzymes) are known and can be measured with satisfactory accuracy. This gives it an advantage over other peptidergic systems in brain such as the encephalins and endorphins, substance P and the pituitary and hypothalamic peptides, releasing factors and release-inhibiting factors. In addition, pharmacological interferences with the renin-angiotensin system are possible at all steps of the enzymatic cascade. This permits optimism as to the progress in brain isorenin-angiotensin research and in resolving conflicting results.

Note Added in Proof:

The separation of renin activity (which forms angiotensin I upon incubation with angiotensinogen) from acid protease activity (which forms product from denatured hemoglobin substrate) has now been accomplished. This enzyme preparation is active in vivo. It increases angiotensin I concentration in CSF and leads to rise of blood pressure if injected into the brain ventricles of conscious rats. The increase of blood pressure can be reversed by angiotensin blockade (G. Speck et al., unpublished observations).

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References


Reply to the Above Letter

Ganten comments on three aspects of the controversy concerning the existence of a brain renin-angiotensin system. These are (1) the properties of the enzyme having renin-like activity in brain, (2) measurement of angiotensin in the brain, and (3) the effects of central administration of angiotensin antagonists. The statements made merit critical comment.

Brain Renin-like Activity

Most investigators have found that the enzyme responsible for renin-like activity in the brain has an optimum pH of 4.5–5.0. Ganten cites the one paper which claims a pH optimum of 7.0–7.5 for the enzyme in dog brain (not rat brain as stated by Ganten). However, the same paper claimed a pH optimum of 7.0–7.5 for kidney renin whose true pH optimum is 6.0 or less, thus casting doubt on the accuracy of the value reported for the brain enzyme. Furthermore, Ganten’s reference to submaxillary gland renin as having a pH optimum of 7.0 cannot be used to infer anything about the enzyme in brain. My statement that the brain enzyme has no measurable activity at pH 7.4 was based on data published by three different groups of investigators (including Ganten). In his letter, Ganten appears to be referring to unpublished work when he states that if he uses enough of the enzyme he does find angiotensin I formation at neutral pH. The immediate question is, of course, how much enzyme, how much angiotensinogen, and how much formation?

Ganten states that I used the low pH optimum of enzyme activity as an argument against its biological role. I was very careful not to use this argument. Indeed, I cited the evidence that the brain enzyme is actually the acid protease cathepsin D which, of course, is active within cell lysosomes, which have a low pH (incidentally, I would not agree with Ganten that in intracellular compartments, "any pH can be realistic"). If angiotensinogen were
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doi: 10.1161/01.RES.42.5.732

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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