Regulation of brain renin: evidence for an independent brain renin

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Summary
1. We examined the effects of various physiological states on systemic and brain renin activities. Activation of the systemic renin–angiotensin system (RAS) by renal ischaemia, captopril or continuous renin infusions had no effect on whole brain or pituitary renin activities. Suppression of circulating renin by bilateral nephrectomy or antirenin antibody injection did not influence brain renin.
2. Brain or pituitary renin activities remained unchanged with various endocrinometabolic disorders such as hypo- or hyper-thyroidism, diabetes and hypophysectomy.
3. Treatment with reserpine or propranolol resulted in significant decrease in brain and pituitary renin levels.
4. We conclude that brain renin activity appears to be generally independent of the systemic RAS. Inhibition of sympathetic activity, however, resulted in suppression of brain renin, suggesting a close interaction between the two systems.

Key words: brain renin, endocrinometabolic disorders, sympathetic nervous system, systemic renin.

Introduction
The existence of brain renin was first suggested by Fischer-Ferraro et al. [1] and Ganten et al. [2]. This has been confirmed recently with improved fractionation techniques and the use of renin-specific antibodies [3–5]. Affinity chromatographic procedures utilizing α-casein, pepstatin or antirenin antibody as ligands successfully separated brain renin from other neutral and acid proteases. Brain renin releases angiotensin I from plasma angiotensinogen at pH 7.4; the pH optimum is 6.0. It has no effect on haemoglobin and its activity is completely neutralized by renin-specific antibody [5]. However, it differs from renal renin by having a lower isoelectric point (pI = 4.82) and a higher $K_m$ (9 x 10^{-4} \text{ mol/l}) for rat plasma angiotensinogen [6]. Brain renin is widely distributed in the brain. The highest concentrations are found in the pineal gland, the choroid plexus and the anterior pituitary gland [6, 7]. The exact function of the brain renin–angiotensin system is unclear. Intraventricular injections of angiotensin II or purified renin resulted in systemic vasopressor response and increase in plasma catecholamines [8–10]. Centrally administered renin or angiotensin II also stimulates thirst, releases antidiuretic hormone and disrupts passive avoidance behaviour [11–12].

In view of the ubiquitous distribution of brain renin, it is also possible that it serves a more universal intracellular function which is as yet undefined. Another important question regarding brain renin is the regulation of its synthesis, activity and degradation.

Characterization of the conditions that stimulate or inhibit brain renin activity may provide additional insight into understanding its function. The purpose of this study is to examine brain renin activity in response to systemic stimuli and physiological conditions known to increase or decrease renal and plasma renin levels.

Methods
Whole brains and anterior pituitary glands of male Sprague–Dawley rats (200–250 g body weight) were removed, homogenized separately in potassium phosphate solution (0.01 mol/l) in sodium chloride (0.1 mol/l), pH 6.5, by a Virtis-60 homogenizer, centrifuged at 12 000 g
for 40 min at 4°C, and then the supernatant was dialysed against an appropriate buffer.

Affinity separation of brain renin from acid protease was accomplished by α-casein chromatography as described before [3, 4]. Brain renin appeared in the unbound fractions.

Angiotensin I generating activity was measured by the method of Haber et al. [13] in the presence of angiotensinase inhibitors (8-hydroxyquinolone, phenylmethylsulphonylfluoride and EDTA) as well 0·2% neomycin at pH 7·4 for 1–24 h. Plasma of nephrectomized rats with no measureable renin activity was used as substrate. Brain renin activity was determined, after separation from acid proteinases by α-casein chromatography, as the angiotensin I generating activity in the unbound fractions [3, 4].

Effect of renin-specific antiserum on the angiotensin I generating activity of various samples was examined as previously described [14]. We examined the effect of various physiological perturbations on whole brain and anterior pituitary renin activities.

**Suppressed plasma renin activity.** (a) Four rats underwent bilateral nephrectomy 48 h before being killed; (b) four rats received an injection of antirenin antibody (100 μl of antirenin IgG, titre 1:20 000) via the tail vein 3 h before they were killed.

**Elevated plasma renin activity.** (a) Five rats received a continuous infusion of purified renal renin, at 4·5 ng of ANG I/h renin activity infused per minute, for 3 days in an Alza osmotic pump implanted intraperitoneally; (b) four rats received intraperitoneal captopril injections (10 μg/g body weight twice daily) for 10 days; (c) the right kidney was made ischaemic in four rats by ligation (90% narrowing) of the abdominal aorta above the right renal artery and below the left. The animals were killed 24 h later.

Inhibition of sympathetic nervous system activity. This was achieved by administration of (a) reserpine subcutaneously (0·125 mg per rat) for 4 days in 10 rats; (b) propranolol subcutaneously (20 mg/100 g body weight in two divided doses) for 5 days in five rats.

Endocrine–metabolic disorders. (a) Five rats were studied 2 weeks after surgical thyroidec-tomy (serum thyroxine <0·25 μg/dl); (b) hyperthyroidism was induced in five rats by daily subcutaneous injections of l-thyroxine (10 μg/100 g body weight) for 10 days (serum thyroxine 54 ± 3 μg/dl); (c) streptozotocin-induced diabetic rats (n = 2) (gift of Dr Hostetter) were examined 4 months after injection; (d) surgically hypothysectomized rats (n = 5) were studied 2 weeks after surgery.

![Fig. 1. Whole brain and anterior pituitary renin activities of rats after various manoeuvres: A, nephrectomy; B, antirenin infusion; C, renin infusion; D, captopril injection; E, renal ischaemia; F, reserpine injection; G, propranolol injection; H, thyroidectomy; I, thyroxine injection; J, streptozotocin-induced diabetes; K, hypophysectomy. *P < 0·05; **P < 0·005, compared with control values.](image_url)

**Results**

The total angiotensin I generating activities of unfractionated whole brain and anterior pituitary extracts in normal rats were 0·1 ± 0·05 and 2·8 ± 0·16 ng of ANG I h⁻¹ mg⁻¹ of protein respectively (n = 6). Isolation of brain renin with an α-casein column yielded renin activities of 1·78 ± 0·13 and 5·4 ± 0·21 ng of ANG I h⁻¹ mg⁻¹ of protein for whole brain and adeno-hypophysis respectively.

The effects of various physiological states on whole brain and anterior pituitary renin activities are shown in Fig. 1. The mean plasma renin activity (PRA) in normal rats was 2·2 ± 0·8 ng of ANG I h⁻¹ ml⁻¹. PRA was undetectable after bilateral nephrectomy or antirenin antibody administration but values were 8·8 ± 1·9, 5·3 ± 1·3 and 12·6 ± 2·9 ng of ANG I h⁻¹ ml⁻¹ in the renin-infused rats, captopril treated animals and those with unilateral renal ischaemia respectively.

Total angiotensin I generating activity of whole brain or anterior pituitary did not change with most of the physiological states examined. Brain renin activities remained unchanged despite marked elevation or suppression of PRA. Brain renin levels were not influenced by changes in endocrine–metabolic states, i.e. hyperthyroidism, thyroidectomy, diabetes or hypophysectomy. However, when rats were treated with reserpine or propranolol, brain renin fell significantly (P < 0·05), as well as pituitary renin levels (P < 0·005).

**Discussion**

Although the various components of the renin–angiotensin system have been demonstrated in...
the brain, little is known about the regulation of brain renin. It is unclear whether brain renin biosynthesis, activity or degradation is under control similar to that of renal renin. Is brain renin activity influenced by systemic stimuli known to increase or decrease renal renin secretion and plasma renin activity? Haulica et al. [15], using bioassay measuring pressor activity, demonstrated that the 'renin-like activity' in extracts of rat pineal and hypophysis decreased with sodium-loading. However, they observed differences in the bioassay pressor response to the tissue eluates and that produced by synthetic angiotensin II. Thus the interpretation of their results was limited by the methodology employed. In this study we examined the effect of elevated or suppressed plasma renin activity on brain renin activity. Neither bilateral nephrectomy nor antirenin antibody administration affected brain renin activity. Depletion of catecholamine stores or propranolol resulted in significant decrease in brain renin activity. Depletion of catecholamine stores or propranolol (β-adrenoreceptor antagonism), whole brain and anterior pituitary renin activities fell significantly, suggesting a causal relationship between inhibition of sympathetic activity and decreased brain renin activity.

The results discussed above suggest that the total brain or pituitary renin activities are not under the same general influence regulation and control as plasma and renal renins. However, the catecholaminergic system appears to influence brain renin activity. Depletion of catecholamine by reserpine and blockade of β-adrenoceptors by propranolol resulted in significant decrease in brain renin activity. The exact nature of the interaction is not clear. One possibility is that central catecholamines play a role in the regulation of the synthesis of brain renin, which may in turn regulate the production of the neuropeptide angiotensin. Alternatively, catecholamines affect the degradation of brain renin. Since we did not examine brain renin activities in various regions, we are unable to address the question of whether there were changes in the regional distribution of brain renin during different physiological states. Taken together, our results argue strongly against contamination of brain extracts by plasma renin as a factor when we measure brain renin activity, and support the existence of an independent brain enzyme. This enzyme, however, appears to be influenced by the catecholaminergic system. The interaction of these two systems may play an important role in the central regulation of systemic blood pressure.

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References
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