Definitive evidence for renin in rat brain by affinity chromatographic separation from protease

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Summary
1. Angiotensin I-generating activity of rat brain extract was separated into two components by affinity chromatography on a casein-Sepharose gel column.
2. The component without affinity to the gel was identified as true renin on the basis of its sensitivity to anti-renin antibody and the lack of protease activity.
3. The second renin-like component with affinity to the gel was a protease insensitive to the anti-renin antibody. Its renin-like activity examined with sheep substrate was pronounced compared with the rate of angiotensin I generation from the rat substrate.
4. It was concluded that rat brain contains true renin, which can be detected by the use of rat substrate but can be masked when examined with sheep substrate.

Key words: affinity chromatography, brain, protease, rat angiotensinogen, renin, sheep angiotensinogen.

Introduction
Renin-like activity has been observed in the brain (Fischer-Ferraro, Nahmod, Goldstein, & Finkelman, 1971; Ganten, Marquez-Julio, Granger, Hayduck, Karsunky, Boucher & Genest, 1971). However, the acidic pH optimum of this enzyme activity and persistent association of this activity with cathepsin led Day & Reid (1976) to suspect that it was due to a non-specific action of acid proteases and that intrinsic brain renin might not exist (Reid, 1977). To strengthen this view Hackenthal, Hackenthal & Hilgerfeldt, (1978) isolated cathepsin from rat brain and demonstrated its renin-like activity. Thus, although the presence and function of all the components of the renin-angiotensin system have been observed in the central nervous system, the nature and identity of the key enzyme, renin in the brain remained unknown.

We have devised an affinity column which can separate cathepsin from renin in one step. Highly specific anti-renin antiserum was used to distinguish renin and cathepsin. We were also able to identify the cause of the confusion involving renin and cathepsin.

Materials and methods
The affinity column for protease used to separate cathepsins from renin was prepared by coupling 800 mg of bovine a-casein (Sigma) to 50 ml of cyanogen bromide-activated Sepharose 4B gel (Pharmacia). Precipitating antibody against pure pig renin (Inagami & Murakami, 1977) had been shown to neutralize rat renin completely (Hirose et al., 1977). Renin-free renin substrate was prepared from plasma of nephrectomized sheep or pooled plasma of nephrectomized rats was used directly. Renin activity was determined by the radioimmunoassay of angiotensin I (Haber, Koerner, Page, Kliman & Purnode, 1969) generated from the substrate incubated with brain extracts. Protease activity was determined by the release of radioactivity from haemoglobin labelled with [14C]glycine methyl ester (Williams & Lin, 1971).

Whole brain obtained from a bilaterally nephrectomized, pentobarbital-anaesthetized, exsanguinated and saline perfused male adult rat was homo-
genized in 40 ml of 0.01M-pyrophosphate buffer (pH 6.5)–saline in a steel-blade homogenizer (VirTis 60), centrifuged at 100 000 g for 45 min and the supernatant was used for subsequent studies.

**Results**

The angiotensin I-generating activity of the crude rat brain extract examined with sheep renin substrate (0.02 μM) was approximately 60-fold greater than the activity determined with rat plasma (0.3 μM) as substrate. Treatment of the extract with 1:30 dilution of rabbit anti-(pig renin) antibody at 4°C overnight caused only slight (less than 20%) inhibition of the angiotensin I-generating activity from the sheep substrate at pH 5.5. At neutral pH the inhibition was negligible. However, when rat plasma was used as substrate, the same antibody treatment inhibited the angiotensin I-generating activity by more than 60% at pH 6.5. These observations suggested that at least 2 different types of enzymes, presumably true renin and protease were responsible for the angiotensin I-generating activity of the brain extract. Recognizing the affinity of proteases to proteins, Sepharose-bound proteins were examined for possible use for affinity chromatographic separation of the two types of renin-like enzymes. Casein–Sepharose gel was found to be effective for such separation (Fig. 1C). The enzyme activity of the first peak was completely neutralized by incubation with the antibody. On the other hand the activity in the second peak was not affected by the same antibody treatment. This enzyme has a strong acid protease activity as indicated by the degradation of [14C]-labelled haemoglobin. The first peak did not show the protease activity (Fig. 1B). The activity of this enzyme to generate angiotensin I from sheep substrate is 60 times higher than true renin (first peak) in the extract. With rat substrate the activity of the true renin is comparable with that of protease (Fig. 1C).

The angiotensin I-generating activity of the first peak determined with the rat substrate is optimal in a neutral pH region between 6 and 7.5, whereas that of the second peak is optimal at pH 4.5 with rat substrate. The level of the renin activity in the brain when assayed with the rat substrate was higher than 200 pg of angiotensin I/h/g wet wt. whereas the plasma renin concentration of the nephrectomized rat was below 50 pg of angiotensin I/h/ml.

**Discussion**

These results indicate that true renin exists in rat brain. The renin-like activity present in the brain could be separated into two clearly different types of enzymes by the present affinity column. The enzyme without affinity can be considered as the true renin since it possesses the following specific properties: 1. sensitivity to the specific anti-renin antibody; 2. lack of the protease activity and 3. neutral pH optimum.

The second enzyme with strong affinity to the casein, with acid protease activity but without sensitivity to the specific anti-renin antibody, could very well be a cathepsin. The angiotensin I-generating activity of the protease is strongly species dependent. Determination of renin-like activity with sheep substrate can easily be masked by the overwhelming contribution of the acid protease. This could be the reason for identifying cathepsin D as the renin-like enzyme in some recent studies. Use of nephrectomized and exhaustively exsanguinated animals and the higher renin activity in the brain tissue compared with the plasma level.
eliminate the possibility that renin activity observed with the present brain extract is due to the contamination by blood renin. Thus, it is clear that rat brain contains true renin in agreement with results independently obtained by Osman, Sen & Smeby (1978) and Dworschack, Gregory & Printz (1978).

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References
