Impermeability of the blood–cerebrospinal fluid barrier for angiotensin II in rats

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Summary
1. Anaesthetized, nephrectomized rats were infused intravenously with unlabelled angiotensin II (AII) or with [3H]angiotensin II ([3H]-labelled AII). The brain ventricular system was perfused with artificial cerebrospinal fluid. The perfusate was collected from the cisterna magna and analysed for AII by radioimmunological and biochemical methods.

2. No increase of immunoreactive AII in cerebrospinal fluid could be shown during intravenous infusion of AII.

3. During intravenous infusions of [3H]-labelled AII at pressor doses small amounts of radioactivity were found in cerebrospinal fluid perfusate.

4. The radioactivity of cerebrospinal fluid outflow could not be related to AII.

Key words: angiotensin II, blood–cerebrospinal fluid barrier, polyacrylamide gel electrophoresis, radioimmunoassay.

Introduction
Centrally administered AII has marked effects on arterial blood pressure, on release of antidiuretic hormone and on water intake (for review see Severs & Daniels-Severs, 1973). These effects are in part mediated by periventricular angiotensin-sensitive brain sites (Johnson & Epstein, 1975).

A pressor substance related to angiotensin was shown in cerebrospinal fluid of man (Finkielman, Fischer-Ferraro, Díaz, Goldstein & Nahmod, 1972).

Abbreviation: AII, angiotensin II.

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To determine whether blood-borne AII can contribute to immunoreactive AII in cerebrospinal fluid, the permeability of the blood–cerebrospinal fluid barrier for AII was studied. Preliminary experiments with intravenous bolus injections of [125I]-labelled AII did not demonstrate penetration of intact AII into cerebrospinal fluid of rats (Ganten et al., 1975). Since [125I]-labelled AII does not correspond to the native AII molecule, the permeability of the blood–cerebrospinal fluid barrier was now investigated with intravenous infusion of [3H]-labelled AII and unlabelled Val4-AII.

Methods
Male Sprague–Dawley rats (mean body weight 240 g) were nephrectomized 16 h before the experiment under urethane anaesthesia (1.25 g/kg intraperitoneally). Under the same anaesthetic the carotid artery and the jugular vein were cannulated for blood pressure recording, blood sampling and continuous infusions of either sodium chloride solution (9 g/l; saline) or Val4-angiotensin II-amide (Hypertensin, Ciba) or [3H]-labelled AII. Blood was collected on ice in plastic vials containing angiotensinase inhibitors (Oster, Hackenthal & Hepp, 1973), centrifuged at 4°C and separated plasma stored at -20°C until assayed. The brain ventricular system was perfused with artificial cerebrospinal fluid at 20 μl/min from the lateral to the fourth ventricle (Ganten et al., 1975). Fractions of the perfusate were collected every 10 min as described for blood. Contamination of this perfusate with blood cells was verified by centrifugation.
reactive AII was measured by direct radioimmunoassay, subtracting blanks obtained from cerebrospinal fluid perfusate and plasma (Oster et al., 1975).

Six rats were infused intravenously during three experimental periods of 30 min each, with saline, with AII (1.2 x 10^{-10} mol min^{-1} kg^{-1}) and with saline again. Three rats were infused intravenously with ³H-labelled AII for 30 min at 3 μCi/min. The specific radioactivity was 24 μCi/nmol. Blood was sampled before the ³H-labelled AII infusion was stopped. The radioactivity was measured by scintillation counting. ³H-labelled AII and the radioactive fragments in plasma and cerebrospinal fluid were separated on a vertical polyacrylamide slab-gel electrophoresis system (Hoefer Instruments) with 20% and 12% gel concentrations and Tris/borate buffer (0.065 mol/l), pH 10.2 (Allen & Moore, 1966). The gel was cut into pieces 0.5 cm in length, put into a toluene/Soluene 350 (Packard) scintillation mixture (10:1) overnight for elution and counted after light-stabilization. ³H-labelled AII and the radioactive fragments separated on the gel were further characterized by radioimmunoassay.

Results

Intravenous infusions of pressor doses of AII into

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**Fig. 1.** (a) Blood pressure of anaesthetized nephrectomized rats during intravenous infusion of AII (at arrows 1.2 x 10^{-10} mol min^{-1} kg^{-1}) or of saline and the corresponding values of immunoreactive AII in plasma and cerebrospinal fluid (CSF). **P < 0.01; * P < 0.05 (n = 6).** (b) Characterization of ³H-labelled AII in buffer (standard) and in plasma and cerebrospinal fluid (CSF) after intravenous infusion of ³H-labelled AII (3 μCi/min). Continuous lines represent the distribution of radioactivity; broken lines represent the distribution of immunoreactivity on the polyacrylamide slab-gel electrophoresis system. Arrows indicate the migration of the Bromophenol Blue marker.
nephrectomized anaesthetized rats did not lead to any increase of immunoreactive AII in cerebrospinal fluid perfusate despite high plasma concentrations (Fig. 1a). The AII concentrations in plasma during the experiments were: 11.4 ± 3.4 × 10⁻¹⁵ mol/ml after saline infusion, 706 ± 81.9 × 10⁻¹⁵ mol/ml after AII infusion and 310 ± 3.3 × 10⁻¹⁵ mol/ml after saline infusion. The mean concentrations of AII in the corresponding samples of cerebrospinal fluid were: 9.8 ± 3.0 × 10⁻¹⁵ mol/ml, 9.7 ± 2.8 × 10⁻¹⁵ mol/ml and 12.2 ± 2.9 × 10⁻¹⁵ mol/ml. However, within the AII infusion period there were marked variations of immunoreactive AII concentrations. The first 10 min fraction (4.2 ± 2.5 × 10⁻¹⁵ mol/ml) contained significantly less immunoreactive AII than the following one (15.5 ± 7.0 × 10⁻¹⁵ mol/ml) (P < 0.05).

The infusion of ³H-labelled AII at 3 μCi/min was associated with a blood pressure increase of 42 mmHg. Radioactivity in cerebrospinal fluid perfusate increased with time. The values for the 10, 20 and 30 min fractions were: 9.36 ± 9.82 nCi/ml, 33.00 ± 1.85 nCi/ml and 61.62 ± 1.51 nCi/ml. After 40 min a further increase to 87.78 ± 2.78 nCi/ml was observed though ³H-labelled AII infusion was terminated. Radioactivity in blood was 1884.8 ± 104.8 nCi/ml. The third fraction of cerebrospinal fluid perfusate contained 3.3% of the radioactivity measured in blood. A typical example of the electrophoretic separation is illustrated in Fig. 1(b). In plasma, three main peaks of radioactivity were shown, representing intact AII, immunoreactive fragments and small non-immunoreactive fragments. Characterization of the cerebrospinal fluid fractions revealed that no intact AII and no immunoreactive fragments were present. The single peak of radioactivity in cerebrospinal fluid was identical with the non-immunoreactive material in plasma. Recovery of the system for ³H-labelled AII was 75%.

**Discussion**

Radioautographic studies have shown an uptake of radioactivity by brain tissue after intravenous injection or infusion of radioactively labelled AII (Volicer & Loew, 1971; Johnson & Epstein, 1975). From the characterization of the radioactivity in brain homogenate and plasma on Dowex columns, Volicer & Loew (1971) concluded that intact ¹⁴C-labelled AII penetrated into the brain. However, they found only one radioactive fraction in plasma and in brain tissue homogenate 5 min after intravenous injection. Since most of the AII is degraded into fragments after this time, no separation of intact AII and AII fragments seems to be obtained on their system.

Small but increasing amounts of radioactivity were shown in cerebrospinal fluid outflow even when the infusion of ³H-labelled AII was stopped (Johnson, 1975; also this report). Circulating ³H-labelled AII is degraded quickly by angiotensinases and most of the radioactivity in plasma consists of small non-immunoreactive AII fragments (Fig. 1b) which may cross the blood–brain barrier. Many amino acids, such as tyrosine, are taken up freely by brain tissue (Oldendorf, 1971). The small amounts of radioactivity in cerebrospinal fluid perfusate consisted exclusively of non-immunoreactive AII fragments. This indicates that only small fragments of AII or amino acids penetrate from blood into cerebrospinal fluid.

Infusion of unlabelled AII did not lead to an increase of immunoreactive AII in cerebrospinal fluid perfusate. However, there is a possibility that small amounts of intact AII might penetrate into the brain ventricular system, which may then be bound or degraded into fragments by brain tissue. Since the antibody used cross-reacts to about 100% with larger AII fragments (hepta-, hexa- and penta-peptide) the immunoreactive material in the outflowing cerebrospinal fluid should nevertheless increase if AII could penetrate into the brain ventricular system. We conclude from our results that neither native AII nor ³H-labelled AII penetrates into cerebrospinal fluid.

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**References**


