Blood pressure effects of endogenous brain angiotensin in rats are increased by inhibition of prostaglandin biosynthesis

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Summary

1. In Sprague-Dawley rats experiments were carried out to evaluate the influence of prostaglandins (PG) on the local generation of angiotensin (ANG) from brain angiotensinogen in cerebrospinal fluid and on the blood pressure effects of brain ANG.

2. In rats, pretreated with the PG biosynthesis inhibitor indomethacin (5 mg/kg subcutaneously every other day for 10 days), hog kidney renin was injected in doses of 0.001, 0.01 and 0.1 unit into the lateral brain ventricle.

3. At 15 min after the renin injections cerebrospinal fluid concentrations of ANG I and ANG II were markedly increased in a dose-dependent manner in the indomethacin-treated and in the untreated groups. At 60 min after the injection of renin ANG I concentrations decreased in both groups. However, the fall in ANG I in cerebrospinal fluid was more marked in indomethacin-pretreated animals.

4. Renin in doses of 0-001, 0-01 and 0-1 unit was injected into the lateral brain ventricles of unanaesthetized normotensive rats. A dose-dependent, long-lasting (>2 h) increase in blood pressure of 9, 15 and 20% was observed. In conscious rats pretreated with indomethacin the blood pressure effects of the renin were greater (12, 23, 27%) when compared with results for untreated controls.

5. Intracerebroventricular injection into conscious rats of the ANG II antagonists [Sar1, Val2, Ala8]ANG II and [NSuc1, Val2, Phg8]ANG II in doses of 1 and 10 μg/kg reduced or abolished the renin-induced increases in blood pressure.

6. The results demonstrate that ANG I as well as ANG II are generated from brain angiotensinogen. The endogenously formed ANG II increases arterial blood pressure. This effect can be inhibited by brain intraventricular administration of specific ANG II antagonists, but it can be increased by inhibition of prostaglandin biosynthesis.

Key words: angiotensin II antagonists, brain, central blood pressure regulation, prostaglandin biosynthesis, renin–angiotensin system.

Abbreviations: PG, prostaglandin; ANG, angiotensin.

Introduction

The central actions of angiotensin (ANG) to increase arterial blood pressure, stimulate thirst, release pituitary hormones and interfere with memory processes are elicited by injection of ANG II directly into the brain (Severs & Daniels-Severs, 1973; Morgan & Routtenberg, 1977). There is an increasing body of evidence for the existence of an endogenous renin–angiotensin system in brain (Ganten & Speck, 1978). All components of this enzyme system necessary to form ANG II have been found in the brain. Besides a renin–angiotensin system in brain, prostaglandins have also been identified in brain, in superfusates of various brain regions and in cerebrospinal fluid (Wolfe, 1975; Wolfe & Coceani, 1979). The biosynthesis of prostaglandins (PG) in rat brain
can be prevented by indomethacin (Wolfe, Rostworowski & Pappius, 1976; Abdel-Halim, Sjöquist & Änggard, 1978). ANG II itself stimulates the synthesis of PG in various systems (McGiff, Crowshaw, Terragno & Lonigro, 1970; Gimbrone & Alexander, 1975). An interaction of the brain renin–angiotensin system with PG seems probable since part of the central actions of ANG, the drinking response, can be suppressed by intracerebroventricular injection of arachidonic acid and PGE 

\[ \text{Fluharty} \text{& Epstein, 1979}. \]

The present experiments in rats were carried out to evaluate the influence of PG on the local generation of ANG from brain angiotensinogen in cerebrospinal fluid and on blood pressure effects of brain ANG.

**Methods**

1. **Blood pressure effects of intracerebroventricular renin**

   Experiments were carried out in male Sprague–Dawley rats, weighing 270–420 g. Implantation of a cannula into the lateral cerebral ventricle and blood pressure measurements were done as described by Mann, Phillips, Dietz, Haebara & Ganten (1978). The single injections were 0-001, 0-01 or 0.1 unit of renin (NBC, Cleveland, Ohio, U.S.A.) in a volume of 5 µl and single injections of the ANG II antagonists [Sar\(^1\), Val\(^2\), Ala\(^8\)]ANG II and [NSuc\(^1\), Val\(^2\), Phg\(^8\)]ANG II were in doses of 1 and 10 µg/kg in a volume of 2.5 µl. For control injections artificial cerebrospinal fluid was used. The period of observation after the injection was 2 h. The rats were awake, unrestrained and freely moving during testing and recording (n = 141).

2. **Enzyme assay in vivo**

   Three to 4 days after implantation of the cannula in the lateral ventricle, rats were anaesthetized with pentobarbital and mounted in a stereotaxic instrument. The membrane atlanto-occipitalis was dissected and a 18 Record needle connected to PE-20 tubing was inserted into the fourth brain ventricle with a micromanipulator. Renin was injected into the ventricle in doses of 0-001, 0-01 or 0.1 unit. Cerebrospinal fluid was drained and collected into plastic vials containing angiotensinase inhibitor placed on ice 15 and 60 min after the injection of renin (n = 104). Angiotensinogen, ANG I and ANG II were determined by radioimmunoassay. In protocol 1 and 2 rats were pretreated with indomethacin (5 mg/kg subcutaneously) every other day for 10 days.

3. **Nephrectomy**

   Bilateral nephrectomy was performed in rats (n = 37) and 16–20 h after nephrectomy renin was given intracerebroventricularly and blood was collected. ANG I, ANG II and renin activity in plasma were determined by radioimmunoassay. Values are given as means ± se of the means. Results were analysed by Student’s paired and unpaired t-test if appropriate, and linear regression analysis. The 5% probability was used as the criterion of significance.

**Results**

Intracerebroventricular injections of renin in doses of 0.001, 0.01 or 0.1 unit induced dose-dependent, long-lasting (>2 h) increases in blood pressure of 9, 15 or 20% (P < 0.01) in unanaesthetized normotensive rats. (Fig. 1) In rats pretreated with indomethacin the blood pressure effects of the renin injections were higher (12, 23, 27%) as compared with untreated controls (P < 0.05). Pretreatment by intracerebroventricular injections of the two different ANG II antagonists [Sar\(^1\), Val\(^2\), Ala\(^8\)]ANG II and [NSuc\(^1\), Val\(^2\), Phg\(^8\)]ANG II in doses of 1 and 10 µg/kg reduced (P < 0.05) or abolished completely the renin-induced blood pressure increases in conscious rats. The injections with
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Artificial cerebrospinal fluid were without influence on arterial blood pressure in conscious rats.

At 15 min after the renin injections concentrations of ANG I and ANG II in cerebrospinal fluid were markedly increased ($P < 0.05$) in a dose-dependent manner in the indomethacin-pretreated and in the untreated groups, and angiotensinogen decreased. The fall in ANG I concentration at 60 min was more marked in indomethacin-pretreated animals: ANG I concentration in cerebrospinal fluid was $1903 \pm 347$ fmol/ml after 0-01 unit of renin in controls, and $452 \pm 119$ fmol/ml in indomethacin-pretreated rats ($P < 0.001$). In bilaterally nephrectomized rats the renin injections were without influence on ANG I, ANG II and renin activity in plasma.

Discussion

Angiotensinogen is present in high concentrations in cerebrospinal fluid (Printz & Lewicki, 1977; Ganten & Speck, 1978). It decreased after intracerebroventricular injection of renin, indicating that ANG I was cleaved from its high-molecular-weight precursor angiotensinogen in cerebrospinal fluid as was also found for dogs (G. Sponer, U. Ganten, P. Schelling & D. Ganten, unpublished work). In nephrectomized rats no change in plasma ANG I, ANG II and renin activity before and after intracerebroventricular injection of renin was observed, indicating that formation of ANG was a local phenomenon. The inhibitory effects of the pretreatment with ANG II antagonists also support this conclusion. The cerebrospinal fluid ANG I and subsequent conversion into ANG II was dose-dependent. At 15 min after the renin injections ANG I and ANG II concentrations in cerebrospinal fluid were similarly increased in the indomethacin-treated and in the untreated groups. At 60 min after the injection of renin ANG I concentrations decreased in both groups, but the fall in ANG I was more marked in indomethacin-pretreated rats. In view of the effects of PG on enzymatic renin activity (Kotchen & Miller, 1974; Zahn & Ganten, 1976) and on renin release (Fröhlich, Hollisfeld, Dormois, Fröhlich, Seyberth, Michelakis & Oates, 1976; Romero, Dunlap & Strong, 1976), it can be concluded that the decreased brain PG concentrations after indomethacin treatment (Wolfe et al., 1976; Abdel-Halim et al., 1978) are involved in the decrease of ANG I in cerebrospinal fluid at 60 min. The stimulation of brain ANG II was followed by a dose-dependent increase in blood pressure after intracerebroventricular renin injections (Reid & Moffat, 1978). Pretreatment with PG biosynthesis inhibitor indomethacin in doses sufficient to inhibit PG biosynthesis in rat brain (Abdel-Halim et al., 1978) significantly increased the blood pressure effects of endogenous brain ANG. In acute experiments in rats meclofenamate injected intracerebroventricularly had no significant effect on the blood pressure response of ANG II injected into the lateral ventricle (Phillips & Hoffman, 1977). However, the augmented central pressor response to ANG II after indomethacin treatment could also be considered as a consequence of the removal of PG inhibition on sympathetic vasoconstrictor activity (Fredholm & Hedqvist, 1975), thus increasing the sensitivity of the peripheral vascular beds to vasoconstrictor substances which are released into the blood after central ANG II stimulation.

References


