Endogenous Angiotensin II Inhibits Production of Cerebrospinal Fluid During Posthypoxemic Reoxygenation in the Rabbit

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Background and Purpose: The choroid plexus, the major source of cerebrospinal fluid (CSF), contains receptors for angiotensin II and a very high concentration of angiotensin converting enzyme. Circulating angiotensin II decreases blood flow to the choroid plexus and the production of CSF. During recovery from hypoxia, marked increases in circulating angiotensin II have been described in some studies. We tested the hypothesis that CSF production decreases during posthypoxic reoxygenation and examined related changes in plasma concentrations of angiotensin II. We also determined whether effect of posthypoxic reoxygenation on production of CSF is due to endogenous release of angiotensin II.

Methods: We measured production of CSF in chloralose-anesthetized rabbits using ventriculocisternal perfusion of artificial CSF containing blue dextran. After control measurements, rabbits were subjected to one of the following interventions: (1) 30 minutes of hypoxia (PaO₂ = 36±1 mm Hg [mean±SE]) followed by 90 minutes of reoxygenation; (2) 30 minutes of hypoxia (PaO₂ = 37±2 mm Hg) followed by 90 minutes of reoxygenation in the presence of the angiotensin II antagonist saralasin; (3) hypoxia for 120 minutes (PaO₂ = 35±1 mm Hg); and (4) infusion of vehicle under normoxic conditions for 120 minutes (time control). Plasma concentrations of angiotensin II were also measured (radioimmunoassay) under control conditions, during hypoxia, and during posthypoxic reoxygenation (first intervention) and at corresponding time intervals in time control animals (fourth intervention).

Results: Under control conditions, the rate of production of CSF averaged 6.7±0.1 μL/min. During posthypoxic reoxygenation, production of CSF decreased by 31±4% (P<.05). In the presence of saralasin, CSF production did not change significantly during posthypoxic reoxygenation (12±6%; P>.05). In time control animals and during prolonged hypoxia, CSF production did not change significantly (12±5% [P>.05] and 9±7% [P>.05], respectively). Plasma concentrations of angiotensin II were below the threshold of sensitivity of the assay under control conditions and during interventions in animals that were made hypoxic and then reoxygenated and in time control animals.

Conclusions: CSF production decreases during posthypoxic reoxygenation. Since plasma concentrations of angiotensin II did not change during posthypoxic reoxygenation, this effect does not appear to be mediated by increases in circulating angiotensin II. We speculate that endogenous release of angiotensin II, perhaps in the choroid plexus epithelium, decreases production of CSF after hypoxic brain injury. (Stroke. 1994;25:1489-1494.)

Key Words: angiotensins • cerebrospinal fluid • choroid plexus • saralasin • rabbits

Hypoxia may occur during the course of diseases of the heart, lungs, and brain. While hypoxic damage involves many physiological and biochemical changes, one of the least explored is the importance of formation of cerebrospinal fluid (CSF) in the pathogenesis of posthypoxic intracranial hypertension. Increased intracranial pressure is a feature of hypoxic brain injury,1 and changes in CSF dynamics and consequent effects on intracranial fluid volume may be factors in the development of intracranial hypertension during or after hypoxia.

Blood vessels of the choroid plexus, which is the main site of formation of CSF,2 have a fenestrated endothelium that allows blood-borne hormones to reach smooth muscle and secretory epithelium.3 Studies by us and others suggest that several vasoactive substances, including endothelin, vasopressin, angiotensin II, and catecholamines, reduce blood flow to the choroid plexus4-7 and reduce CSF production.8-12 Hypoxia has significant effects on the renin-angiotensin system. Activity of angiotensin converting enzyme is reduced13-15 and plasma levels of angiotensin II decrease during acute hypoxia.16-18 In contrast, angiotensin II levels have been reported to increase greatly during posthypoxic reoxygenation in sheep.17 Thus, changes in endogenous levels of angiotensin II during and after hypoxia may influence the rate of CSF production and the development of intracranial hypertension.

The goal of this study was to examine the influence of endogenous angiotensin II on CSF production during posthypoxic reoxygenation. Specifically, we tested the hypothesis that CSF production decreases during posthypoxic reoxygenation. We also determined whether this decrease is mediated by angiotensin II.

Materials and Methods

General Preparation of Animals

The experimental protocol for this study was approved by the Animal Care and Use Committee of the University of

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Iowa. Thirty-one New Zealand White rabbits (weight, 2.0 to 3.4 kg) were used in the experiments. The animals had free access to food and water until the time of surgical preparation for the experiments. They were anesthetized with sodium thiopental administered via an ear vein catheter (25 to 30 mg · kg⁻¹ · h⁻¹) by intermittent boluses of 0.8 to 1.0 mg · kg⁻¹ · IV). After infiltration of the skin of the neck with bupivacaine 0.5%, a tracheostomy was performed. The administration of sodium thiopental was stopped, and chloralose was administered (30 to 40 mg · kg⁻¹ · IV followed by 10 to 20 mg hourly throughout the duration of the experiment). Gallamine triethiodide (5 mg · kg⁻¹ · IV) was administered after induction of anesthesia, followed by intermittent boluses of 1.5 mg · kg⁻¹ · h⁻¹ to maintain muscle relaxation as needed. Throughout each experiment, depth of anesthesia was assessed by noting the blood pressure change to periodic pressure on the tail. Additional chloralose (10 to 20 mg IV) was administered if a change in blood pressure occurred. The animals were mechanically ventilated with air and supplemental oxygen and nitrogen (FiO₂=0.5) to maintain a Paco₂ of approximately 35 to 45 mm Hg and PaO₂ above 100 mm Hg. A catheter was inserted into a femoral artery (after skin infiltration with bupivacaine) to monitor blood pressure and to sample arterial blood. A catheter was also inserted into a femoral vein for administration of fluids and drugs. Arterial blood gases were monitored two to three times during the surgical preparation and at 45-minute intervals thereafter. The partial pressures of inspired oxygen and end-tidal carbon dioxide were monitored constantly during the studies (Datex 254 Airway Gas Monitor, Puritan Bennett). Rectal temperature was monitored and maintained at 37°C to 38°C with a heating pad. Lactated Ringer's solution was infused at 7 to 10 mL·kg⁻¹·h⁻¹ to maintain hydration.

Production of CSF
To measure the rate of CSF production, we used ventriculocisternal perfusion as described previously in detail.⁸⁻¹¹ The animal's head was fixed in a stereotaxic holder, and a 20-gauge needle was placed stereotaxically into one lateral ventricle. The location of the lateral ventricle was confirmed during the insertion of the needle when the pressure, measured through a side port, abruptly decreased and an appropriate CSF pressure wave pattern varying with heartbeat and respiration was identified. After exposure of the atlanto-occipital membrane, the tip of a 23-gauge "butterfly" needle was placed in the cisterna magna, and the plastic hub of the catheter was adjusted to the level of the auditory meatus. To prevent leakage around the cisternal needle, Super Glue was applied around the insertion site. Artificial CSF⁵ was infused into the lateral ventricle at 30 μL·min⁻¹ using a gas-tight syringe while infusion pressure was measured through a side port. The perfusion fluid contained 1 mg·mL⁻¹ of the nondiffusible indicator blue dextran (molecular weight, 2×10⁶). Success of ventriculocisternal perfusion was subsequently confirmed by the appearance of the blue dye and the absence of blood in the effluent that drained through the cisternal catheter. The effluent was collected continuously and divided into aliquots at 15-minute intervals. If obstruction of the cisternal needle occurred, as evidenced by a gradual increase in CSF pressure and a pattern of decreased volume of the collected perfusate, the experiment was discontinued. If leakage of perfusate was noted around the cisternal needle (which was evidenced by a decrease in the measured CSF pressure and decrease in collection of cisternal perfusate), the experiment was also discontinued. The concentration of blue dextran in the infusion fluid was measured with a spectrophotometer (wave length, 610 nm), and the rate of production of CSF in microliters per minute was calculated from the dilution of blue dextran with the formula:

\[ V_v = \frac{V_i(C_v - C_o)}{C_o} \]

where \( V_v \) is the rate of production of CSF, \( V_i \) is the rate of infusion of perfusion fluid, and \( C_v \) and \( C_o \) are the concentrations of blue dextran in the inflow and outflow fluids, respectively.

Experimental Protocols of CSF Production Experiments
In all studies, ventriculocisternal perfusion was performed for 4.5 hours. The first 2 hours provided adequate time for equilibration of the tracer to be reached. A timer was then reset to zero. If a stable value for the rate of production of CSF was not reached during the first 2 hours of ventriculocisternal perfusion, the results of the experiment were discarded. In all groups, the cisternal outflow aliquots collected between 0 and 15 minutes and between 15 and 30 minutes after the end of the equilibration period were used to determine the first and second control measurements of CSF production, respectively. After the second control measurement, the intervention to be tested was performed and ventriculocisternal perfusion continued for another 120 minutes. Cisternal perfusates collected at 30, 90, and 120 minutes. In four of these animals, four blood samples (2 mL each) were taken for measurement of plasma angiotensin II concentration by radioimmunoassay, as described previously.²⁰ The first blood sample was taken between the first and second control CSF production measurements. The three subsequent blood samples were taken 30 minutes after the onset of hypoxia and 5 and 90 minutes after resuming reoxygenation.

In group 2 (n=7), CSF production was measured as described in group 1, except that an infusion of the angiotensin II antagonist saralasin (1 μg·kg⁻¹·min⁻¹) was started just before reoxygenation and continued to the end of the experiment. The goal of this group was to examine whether the decrease in CSF production during posthypoxic reoxygenation was due to stimulation of angiotensin II receptors. We used this dose of saralasin because it completely inhibits the decrease in blood flow to the choroid plexus and the production of CSF in response to angiotensin II⁹ (see "Discussion").

In group 3 (n=7), after control measurements, hypoxia was induced and maintained for 120 minutes without reoxygenation (prolonged hypoxia). The goal of this group was to examine the effect of prolonged hypoxia on CSF production over the entire experimental time period (hypoxia time control).

In group 4 (n=7), after control measurements, no intervention was made, and lactated Ringer's was infused for 120 minutes. This group served as a time control to verify the stability of CSF production measurements during the time period of this experiment. In four of these animals, four blood samples were taken for measurement of plasma angiotensin II concentrations. The first blood sample was taken between the first and second control CSF production measurements. The three subsequent blood samples were taken at time intervals that are similar to those in the first group of animals: 30, 35, and 120 minutes after the second control CSF production measurements.

Statistical Analysis
ANCOVA was used to compare the effects of the various interventions on CSF production 120 minutes after the beginning of interventions. P₀₂ during the intervention was the covariant factor, ie, PaO₂ during 30 minutes of hypoxia after...
the control period in groups 1 and 2 and the corresponding periods in groups 3 and 4. If the calculated F value was greater than the critical value for the .05 probability level, then pairwise comparison between groups was performed with Fisher's least significant difference test. If the calculated F value was greater than the critical value for the .05 probability level, then Dunnett's t test was used to compare rates of CSF production during the control period to each other and to compare the rates of CSF production at 90, 105, and 120 minutes after the beginning of each intervention to the second control observation. P<.05 was considered significant.

Repeated measures ANOVA was performed to determine whether significant changes in CSF production occurred within each group. If the calculated F value was greater than the critical value for the .05 probability level, then Dunnett's t test was used to compare rates of CSF production during the control period to each other and to compare the rates of CSF production at 90, 105, and 120 minutes after the beginning of each intervention to the second control observation. P<.05 was considered significant.

**Results**

**Arterial Oxygen Partial Pressure (Pao2)**

During hypoxia, Pao2 values were 36±1, 37±2, and 35±1 mm Hg in the animals that were subjected to 30 minutes of hypoxia, 30 minutes of hypoxia in the presence of saralasin, and 120 minutes of hypoxia, respectively. These values were not significantly different (P>.05 [mean±SE]).
hypoxia followed by reoxygenation, prolonged hypoxia alone does not alter the production of CSF.

Consideration of the Method

We and others have measured the rate of production of CSF using ventriculocisternal perfusion. This technique requires the use of a nondiffusible indicator and is widely used in the studies of CSF production. Determination of the rate of production of CSF with this method is based on the dilution of artificial CSF containing blue dextran by CSF produced in the ventricular system of the brain. The rates of production of CSF in this study were within the range previously reported for rabbits (5 to 12 \( \mu \text{L} \cdot \text{min}^{-1} \)).

Hypoxia and the Renin-Angiotensin System

The juxtaglomerular apparatus is one of the sites that produces renin, which converts angiotensinogen to angiotensin I. Angiotensin I is converted to angiotensin II by angiotensin converting enzyme, which is present in endothelium and other cells. Angiotensin II plays a major role in the regulation of renal blood flow and body fluid and osmotic balance.

During acute hypoxia (\( \text{P}_{\text{ao}}_2 \) values of 33 to 36 mm Hg) in sheep, activity of angiotensin converting enzyme is inhibited and plasma levels of angiotensin II decrease. This is similar to the level of hypoxia achieved in this study (\( \text{P}_{\text{ao}}_2 \)=35 to 37 mm Hg). Several additional lines of evidence suggest that the activity of angiotensin converting enzyme is reduced during hypoxia. Furthermore, renal renin content as well as plasma renin activity increase during hypoxia. During reoxygenation, plasma concentrations of angiotensin II quickly reach very high levels in sheep (approximately 1800 pg/mL). Removal of the inhibition of angiotensin converting enzyme by reoxygenation may account for these abrupt large increases in plasma levels of angiotensin II.

In contrast to the previous findings in sheep, plasma concentrations of angiotensin II did not increase during posthypoxic reoxygenation in the rabbit in this study. The discrepancy in these findings may be due to the difference in species. We previously reported that increases in plasma levels of angiotensin II to 427 and 1360 pg/mL decrease blood flow to the choroid plexus by 28% and 47%, respectively. Thus, blood flow to the choroid plexus decreases markedly at these plasma concentrations of angiotensin II. In this study our finding that concentrations of circulating angiotensin II did not increase during posthypoxic reoxygenation is supported by the preliminary finding that blood flow to the choroid plexus did not decrease under the same conditions in a study that we are currently conducting in our laboratory (M.A.M., unpublished data, 1994). However, we cannot rule out the possibility that plasma levels of angiotensin II increased to levels that did not exceed the sensitivity of the assay (50 pg/mL).

The Choroid Plexus and the Renin-Angiotensin System

In addition to the circulating renin-angiotensin system, a local renin-angiotensin system appears to be present in the brain and within the choroid plexus. The choroid plexus contains a high concentration of renin, angiotensinogen, an extremely high concentration of angiotensin converting enzyme, and specific binding sites for angiotensin II. We recently reported that both intravascular and central angiotensin I and II selectively decrease blood flow to the choroid plexus. Circulating and centrally administered angiotensin II also decreases production of CSF. In the present study decreases in CSF production during posthypoxic reoxygenation were not associated with increases in plasma angiotensin II concentrations. However, an angiotensin receptor antagonist inhibited this decrease in CSF production, suggesting that the effect was mediated by endogenous release of angiotensin II.

In conclusion, production of CSF decreases during posthypoxic reoxygenation. Despite the absence of associated detectable increases in plasma concentrations of angiotensin II, this decrease was prevented by systemic blockade of angiotensin II receptors. Thus, we speculate that endogenous release of angiotensin II, perhaps locally in the choroid plexus epithelium, decreases production of CSF after hypoxic brain injury.

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