Effect of Endothelin on Production of Cerebrospinal Fluid in Rabbits

Kimberly A. Schalk, PhD; Frank M. Faraci, PhD; and Donald D. Heistad, MD

Background and Purpose: Endothelin is a potent vasoconstrictor in several tissues, including the choroid plexus. The goal of this study was to determine whether endothelin affects the production of cerebrospinal fluid.

Methods: Ventriculocisternal perfusion was used to measure the production of cerebrospinal fluid in anesthetized rabbits. Changes in production of cerebrospinal fluid were examined in response to vehicle, intravenous endothelin (alone and in the presence of indomethacin), and intraventricular endothelin.

Results: Under control conditions, the reduction in production of cerebrospinal fluid in response to endothelin administered intravenously was only modestly greater than that during infusion of vehicle. Because endothelin releases cyclooxygenase products that attenuate the direct effects of endothelin in several tissues, effects of endothelin on the production of cerebrospinal fluid were also examined after inhibition of cyclooxygenase. Production of cerebrospinal fluid in response to 1 μg/kg i.v. endothelin was reduced more in animals treated with indomethacin than in untreated animals (−34±7% [mean±SEM] versus −14±6%, p<0.05). Thus, effects of endothelin on the production of cerebrospinal fluid are attenuated by cyclooxygenase products. Finally, responses to intraventricular endothelin were examined. Intraventricular endothelin produced a modest, but significant, reduction in the production of cerebrospinal fluid.

Conclusions: In summary, endothelin may play a role in regulation of the brain fluid balance by affecting the rate of production of cerebrospinal fluid, and this effect is modulated by cyclooxygenase products. (Stroke 1992;23:560-563)

Key Words • choroid plexus • cerebrospinal fluid • indomethacin • rabbits

Endothelin is a recently discovered peptide that is synthesized and released from endothelial cells.1,2 Endothelin is also produced by other cells, including neurons, glial cells, and epithelial cells.3-7 Endothelin is an extremely potent vasoconstrictor that also appears to have a major influence on renal function.8 The choroid plexus is functionally similar to the kidney with regard to its secretory activity and its role in fluid volume regulation.9 There is a relatively high density of receptors for endothelin in the choroid plexus,10,11 and we have shown that endothelin produces marked decreases in blood flow to the choroid plexus.12 Because blood flow to the choroid plexus appears to be an important determinant of cerebrospinal fluid (CSF) production,13 we anticipated that endothelin may decrease the rate of production of CSF. The first goal of this study was to test the hypothesis that endothelin decreases the production of CSF.

Endothelin releases cyclooxygenase products, which modulate the direct effects of endothelin.14-17 The second goal of this study was to determine whether cyclooxygenase products modulate the response of the choroid plexus to endothelin.

Very recent studies indicate that endothelin is produced by cells in the brain.3-5 The choroid plexus may, therefore, be exposed to endothelin in CSF as well as endothelin from endothelium and blood. The third goal of our study was to determine whether intraventricular endothelin affects the production of CSF.

Materials and Methods

New Zealand White rabbits (n=68, weighing 3-4 kg) were used. The rabbits were anesthetized with 30 mg/kg i.v. sodium pentobarbital, and supplemental anesthetic was administered continuously (10–15 mg/kg/hr i.v.) for the duration of each experiment. The trachea was cannulated, and the rabbit was ventilated mechanically with room air that was supplemented with oxygen. Paralysis of skeletal muscle was produced with 5 mg/kg i.v. gallamine triethiodide.

The femoral veins were cannulated for the infusion of drugs and supplemental anesthetic. The femoral arteries were cannulated to monitor arterial blood pressure, to withdraw arterial blood samples for measurement of blood gases and pH, and to withdraw blood to maintain systemic blood pressure constant following the infusion of endothelin.
TABLE 1. Effect of Blood-Borne and Intracerebroventricular Endothelin on Production of CSF in Rabbits

<table>
<thead>
<tr>
<th>Endothelin dose</th>
<th>n</th>
<th>Control period (µl/min)</th>
<th>Endothelin (µl/min)</th>
<th>Arterial blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CSF production</td>
<td></td>
<td>Control period</td>
</tr>
<tr>
<td>0.01 µg/kg i.v.</td>
<td>10</td>
<td>6.9±0.4</td>
<td>6.5±0.4</td>
<td>83±1</td>
</tr>
<tr>
<td>0.1 µg/kg i.v.</td>
<td>8</td>
<td>6.6±0.4</td>
<td>5.5±0.5*</td>
<td>87±4</td>
</tr>
<tr>
<td>1 µg/kg i.v.</td>
<td>6</td>
<td>6.4±0.7</td>
<td>5.6±0.7*</td>
<td>82±4</td>
</tr>
<tr>
<td>1 µg intraventricularly</td>
<td>9</td>
<td>7.2±0.4</td>
<td>6.0±0.6*</td>
<td>87±3</td>
</tr>
</tbody>
</table>

Mean±SEM values for blood pH, Pco₂, and Po₂ were 7.45±0.01, 32±1 mm Hg, and 155±5 mm Hg, respectively, before the systemic injection of vehicle or endothelin and did not change during the experiment in any group except for a modest increase in Po₂ in the group that received 1 µg/kg i.v. endothelin.

Mean±SEM values for blood pH, Pco₂, and Po₂ were 7.44±0.01, 34±1 mm Hg, and 184±12 mm Hg, respectively, before the intraventricular injection of vehicle or endothelin and did not change during the experiment.

Production of CSF was measured using a ventriculocisternal perfusion technique similar to that described previously. The rabbits were placed in a head holder in the sphinx position. A burr hole was drilled over the right parietal cortex 1.0 cm caudal to the bregma and 0.75 cm lateral to the sagittal suture. A 25-gauge needle was lowered 0.7 cm through the burr hole into the lateral ventricle using a micromanipulator. A double-bore needle was used for the experiments involving intraventricular injections. A catheter was inserted into the cisterna magna.

Artificial CSF containing a nondiffusible dye (blue dextran, MW 2 million; 1 mg/ml) was infused into the lateral ventricle at a rate of 10 µl/min. The effluent was collected from the cisterna magna at 10-minute intervals with a fraction collector, and the concentration of dye in the CSF that was infused and collected was measured using a spectrophotometer. Artificial CSF containing dye was infused for 120 minutes prior to the control measurements to allow sufficient time for the dye to diffuse throughout the CSF and achieve a steady-state concentration. The rate of CSF production (CSFP) was calculated as CSFP = Cf - Cg/Cc, where C = concentration of dye in infused CSF, and Cg = concentration of dye in collected CSF. A standard curve using known concentrations of blue dextran was prepared for each experiment.

After ventriculocisternal perfusion was initiated, an equilibration period of 120 minutes was followed by a 30-minute control period. The first series of rabbits then received an intravenous injection of 0.01 (n=10), 0.1 (n=8), or 1 (n=6) µg/kg endothelin (endothelin-1, Peninsula Laboratories, Belmont, Calif.) or saline vehicle (n=8).

The second series of rabbits was pretreated with 4 mg/kg i.v. indomethacin 30–40 minutes before the intravenous injection of 0.1 (n=8) or 1 (n=5) µg/kg endothelin or saline vehicle (n=6). We have shown that this dose of indomethacin produces selective inhibition of responses of the cerebral vessels to arachidonic acid. Elevations in blood pressure after the injection of 0.1 or 1 µg/kg endothelin were prevented by withdrawal of blood from an arterial catheter. In several rabbits that received endothelin, systemic blood pressure began to decrease during the last 30 minutes of the experiment. Thus, to maintain systemic blood pressure constant, anticoagulated autologous blood was infused into these rabbits. The response to endothelin in these rabbits was similar to that in rabbits in which blood pressure remained elevated for the duration of the experiment. Thus, the data were combined.

The second series of rabbits was pretreated with 4 µg/kg i.v. indomethacin versus 5.4±0.3 µl/min during the last 30 minutes of the experiment. Thus, to maintain systemic blood pressure constant, anticoagulated autologous blood was infused into these rabbits. The response to endothelin in these rabbits was similar to that in rabbits in which blood pressure remained elevated for the duration of the experiment. Thus, the data were combined.

The third series of rabbits received an intravenous injection of 1 µg endothelin (n=9) or saline vehicle (n=8) into a lateral ventricle after the 120-minute equilibration period. Endothelin was injected in 100 µl artificial CSF (containing 1 mg/ml blue dextran) into the lateral ventricle at a rate of 10 µl/min for 10 minutes.

The average value for CSF production during the 30-minute control period was compared with the average value during the last 30 minutes of collection of CSF (beginning 60 minutes after the injection of endothelin or vehicle), using Student's paired t test; p<0.05 was considered significant. To test the effect of endothelin with or without indomethacin, one-way analyses of variance were performed on the change from control values.

Results

The low dose of endothelin did not change systemic blood pressure. The moderate and high doses of endothelin produced a transient depressor response (decrease in pressure of 2–6 mm Hg) that lasted for approximately 1–2 minutes, followed by an increase in pressure. The increase in blood pressure in response to the moderate and high doses of endothelin was prevented by removal of arterial blood (Tables 1 and 2).

Production of CSF tended to decrease slightly during the experiment in rabbits that received vehicle (6.0±0.2 µl/min during the control period versus 5.3±0.2 µl/min during the last 30 minutes of vehicle injection, p>0.05). The low dose of endothelin did not affect CSF production significantly (Table 1). The moderate and high doses of endothelin produced only small decreases in CSF production (Figure 1 and Table 1). There was no significant effect of endothelin dose.

In rabbits pretreated with indomethacin, the production of CSF tended to decrease during the experiment but, as in the vehicle-treated group that did not receive indomethacin, this change was not statistically significant (5.9±0.4 µl/min during the control period after indomethacin versus 5.4±0.3 µl/min during the last 30 minutes of vehicle injection, p>0.05). After indomethacin, the response to the moderate dose of endothelin (-16±3%) was not different from that without indomethacin.
After indomethacin, the response to the high dose of methacin (-17±4%, Figure 1 and Tables 1 and 2). After indomethacin, the response to the high dose of endothelin (-34±7%, p<0.05; Figure 1 and Tables 1 and 2).

The production of CSF did not change in rabbits in which vehicle was injected into the cerebral ventricles (6.9±0.9 μl/min during the control period versus 6.6±1.1 μl/min during the last 30 minutes of vehicle injection, p>0.05). Intracerebroventricular injection of endothelin decreased the production of CSF by 17±6% (Table 1).

**Discussion**

There are two new findings in this study. First, both blood-borne and intraventricular endothelin decrease the rate of production of CSF. Second, cyclooxygenase products attenuate the response of the choroid plexus to high doses of blood-borne endothelin.

Endothelin has several important effects on the kidney. Endothelin decreases renal blood flow and the glomerular filtration rate and may affect renal Na+ reabsorption.

The choroid plexus is functionally similar to the kidney with regard to secretory activity and volume regulation. Several substances that decrease blood flow to the choroid plexus also decrease the rate of formation of CSF. We have shown previously that endothelin produces marked decreases in blood flow to the choroid plexus. Thus, we anticipated that endothelin may decrease the rate of production of CSF. Endothelin produced a modest, but significant, reduction in the production of CSF.

**TABLE 2. Effect of Blood-Borne Endothelin after 4 mg/kg i.v. Indomethacin in Rabbits**

<table>
<thead>
<tr>
<th>Endothelin dose</th>
<th>n</th>
<th>Control period</th>
<th>Endothelin</th>
<th>Arterial blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 μg/kg i.v.</td>
<td>8</td>
<td>6.1±0.3</td>
<td>5.2±0.4*</td>
<td>94±3</td>
</tr>
<tr>
<td>1 μg/kg i.v.</td>
<td>5</td>
<td>6.1±0.1</td>
<td>4.0±0.5*</td>
<td>80±5</td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid. Values are mean±SEM.

 Activation of endothelin receptors is linked to phosphoinositide hydrolysis in other tissues. Serotonin activates the hydrolysis of phosphoinositides in the choroid plexus and decreases the production of CSF. It is not known, however, if this second messenger mediates the response to endothelin in the choroid plexus.

Endothelin decreased the production of CSF when injected into the cerebral ventricles. Thus, endothelin may also affect the production of CSF when endothelin is released from the cerebral parenchyma.

Endothelin can release cyclooxygenase products from cultured cells and isolated tissue. Indomethacin potentiates the physiological actions of endothelin in several systems. Thus, the direct action of endothelin may be modulated by an endothelin-induced release of cyclooxygenase products. The finding that endothelial denudation or indomethacin augments the vasoconstrictor response to endothelin suggests that endothelium is a major source of vasodilator prostanoids that are released by endothelin.

We considered the possibility that endothelin releases cyclooxygenase products, which attenuate the direct effect of endothelin on the production of CSF. The high dose of endothelin in the absence of cyclooxygenase blockade produced an only modest reduction in the production of CSF. The same dose of endothelin after indomethacin produced a marked reduction in the production of CSF. Thus, it appears that endothelin releases cyclooxygenase products such as prostacyclin or prostaglandin E2, which attenuate the direct effect of endothelin to decrease the production of CSF. Cyclooxygenase blockade did not appear to potentiate the effect of the moderate dose of endothelin, which suggests that high doses of endothelin may be necessary to elicit the release of cyclooxygenase products.

Baseline values for the production of CSF were lower in this study than in a previous study. The reason for this difference is not clear. The lower baseline values in the present study work against our hypothesis that endothelin reduces CSF production. The lower baseline values, however, did not prevent us from detecting a reduction in the formation of CSF, especially in the presence of indomethacin. It is possible that even greater decreases in the production of CSF would be observed in response to endothelin if baseline levels of CSF formation were higher.

In the absence of indomethacin, the reduction in the production of CSF in response to endothelin was only modestly greater than during the infusion of vehicle. Thus, the effects of endothelin on the production of CSF do not appear large under control conditions. A major finding of the present study is that endothelin may have important effects on CSF production, but the
most prominent effect is seen only after inhibition of cyclooxygenase. It seems likely that most responses to endogenous endothelin result from local release of the peptide to adjacent cells. The local concentration of endothelin is not known, but it may be very high. Therefore, we administered endothelin in doses of up to 1 μg/kg to mimic these concentrations. This approach has been used in a number of studies.

Norepinephrine, arginine vasopressin, and serotonin decrease CSF production by approximately 30–40%. Thus, the decrease in CSF production of approximately one third in response to endothelin after indomethacin is similar to the reduction produced by other endogenous inhibitors of formation of CSF.

The choroid plexus may be exposed to endothelin from several sources. Endothelin is produced by glial cells, neurons, and cerebral endothelial cells. Epithelial cells from several tissues produce endothelin, and endothelin immunoreactivity has been described in the choroid plexus. Because the choroid plexus is highly vascular, there is a relatively high concentration of endothelial cells that serve as a potential source of endothelin. Cyclooxygenase products appear to play a negative feedback role on the effect of endothelin on the production of CSF. This may be an important mechanism to antagonize the potent, long-acting effects of endothelin.

Endothelin is released from endothelial cells in response to several stimuli including increases in shear stress and thrombin. Shear stress may increase as blood flow increases during acute hypertension. Intracranial hemorrhage may result in elevated thrombin concentrations in CSF. In patients with cerebrovascular disease, endothelin levels are approximately 20-fold greater in CSF than in plasma. We speculate that, under these conditions, endothelin may serve a protective function by reducing the production of CSF and thereby reducing intracranial pressure.

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References

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