The Cerebrovascular Effects of Curare and Histamine in the Rat

Richard Vesely, M.D.,* William E. Hoffman, Ph.D.,† Katherine S. L. Gill, M.D.,† Ronald F. Albrecht,‡ David J. Miletich†

The effects of histamine and curare on cerebral blood flow (CBF) were measured in rats with an intact blood-brain barrier (BBB) and in rats in which the BBB was disrupted by hypertonic urea. Using radioactive microspheres cortical and subcortical CBF were measured in paralyzed ventilated rats anesthetized with 70% N₂O, 30% oxygen. Blood gas tensions were controlled by mechanical ventilation. In rats with an intact BBB, neither histamine infusion (10 μg·kg⁻¹·min⁻¹) nor curare (1 and 5 mg/kg) increased CBF. Twenty minutes after the BBB was disrupted by 2 M urea, histamine (10 μg·kg⁻¹·min⁻¹) produced an increase in cortical (180–210 ml·100 g⁻¹·min⁻¹) and subcortical CBF (103 to 124 ml·100 g⁻¹·min⁻¹). Twenty minutes after BBB disruption, curare also produced a significant increase in cortical CBF (1 mg/kg: 176–201 ml·100 g⁻¹·min⁻¹, 5 mg/kg: 190–209 ml·100 g⁻¹·min⁻¹). The increases in CBF produced by curare were completely blocked by pretreatment with 30 mg/kg cimetidine, a histamine H₂ receptor antagonist, 3 min before curare. The results indicate that curare may produce cerebrovasodilatation and increases in CBF by release of histamine and stimulation of central nervous system H₂ receptors. These effects occur only when the BBB is disrupted and circulating histamine has access to brain perivascular tissue. (Key words: Antagonists: cimetidine. Blood pressure: drug effects. Brain: blood flow; intracranial pressure; blood-brain barrier. Histamine. Neurumuscular relaxants: d-tubocurarine; pancuronium.)

THE USE OF CURARE IN NEUROANESTHESIA IS CONTROVERSIAL PRIMARILY BECAUSE OF THE DRUG’S POTENT HISTAMINE-RELEASEING PROPERTY.¹ HISTAMINE MAY LOWER BLOOD PRESSURE AND INCREASE INTRACRANIAL PRESSURE (ICP), THUS LOWERING CEREBRAL PERFUSION PRESSURE.² HISTAMINE MAY INCREASE CEREBRAL BLOOD FLOW (CBF) BY A DIRECT CEREBROVASODILATING EFFECT.³⁻⁵ ACCESS OF HISTAMINE TO THE BRAIN MAY BE AN IMPORTANT REQUIREMENT FOR ITS CEREBROVASODILATING EFFECT, AS VASODILATION OCCURS ONLY AFTER THE BLOOD–BRAIN BARRIER (BBB) HAS BEEN DISRUPTED.⁶⁻⁷ H₁ AND H₂ HISTAMINE RECEPTORS ARE KNOWN TO EXIST IN THE CEREBRAL VASCULATURE AND INFLUENCE CEREBROVASCULAR RESISTANCE, WITH THE H₂ RECEPTOR PRIMARILY INVOLVED IN VASODILATION.⁶⁻⁸ IT WAS THE PURPOSE OF THIS INVESTIGATION TO DETERMINE WHETHER INTRAVENOUS HISTAMINE INFUSION AND HISTAMINE RELEASED BY CURARE INJECTION CAN INCREASE CBF IN THE NORMOXIC, NORMOCARBIC RAT. THE ROLE OF THE BBB IN HISTAMINE INDUCED CEREBROVASODILATION WAS ALSO EXAMINED BY THE USE OF CEREBROVASCULAR OSMOTIC SHOCK. LASTLY, THE EFFICACY OF CIMETIDINE IN BLOCKING INCREASES IN CBF FROM CURARE WAS EVALUATED.

METHODS

SURGICAL PREPARATION

Six-month-old male Sprague-Dawley rats were anesthetized with halothane in a bell jar. Following tracheotomy, they were ventilated with 1.5% halothane in oxygen (FIO₂ = 100%) using a Harvard small animal respirator. Rectal temperature was measured with a Yellow Springs Instruments thermistor probe and maintained at 37°C using overhead heat lamps. PacO₂ was maintained at 35–40 mmHg and PacO₂ above 100 mmHg. After bilateral cutdowns, both femoral arteries and one femoral vein were cannulated with heparinized, saline-filled catheters to permit continuous heart rate and blood pressure monitoring, blood withdrawal, and drug administration. The left ventricle was catheterized via the right common carotid artery for radioactive microsphere injections. This technique produced little change in hemispheric CBF under control conditions.⁸ The right common carotid artery was also catheterized in a cephalic direction with a second catheter using a modification of the technique of Harder.¹⁰ The external carotid artery was ligated to permit urea infusions directly into the right internal carotid artery. After completion of the surgery, halothane was discontinued and the animal administered 70% nitrous oxide, 30% oxygen, and iv pancuronium (1 mg/kg) for a 45-min equilibration period.

DRUG TREATMENT

Experiments were carried out in two parts using a total of 63 rats. In the first part, rats were tested with iv histamine infusion with an intact or disrupted BBB to evaluate the ability of histamine to increase CBF. Group 1 (n = 10) received 1 ml of 2 M urea infused into the right internal carotid artery over 30 s to disrupt the BBB. Group 2 (n = 8) received an infusion of 1 ml of normal saline in place of urea. CBF was measured in both groups under three separate test conditions. These are described graphically in figure 1. The first test, made with cobalt-
57 microspheres, was given 10 min after an intracarotid urea or saline infusion. In the second test, each rat was given an iv histamine (histamine phosphate) infusion of 10 µg·kg⁻¹·min⁻¹ which decreased mean arterial blood pressure (MABP) approximately 25 mmHg. After a 5-min infusion, a second CBF measurement was obtained using tin-113 labeled microspheres. A final CBF measurement was made 30 min after completion of the histamine infusion using scandium-46 labeled microspheres.

A second experiment was carried out to determine whether histamine, released endogenously by curare injection, may also increase CBF. Separate rats were used for this experiment. The methods used in the second experiment were similar to the first, in that all rats received three microsphere injections (CBF measurements). One group of rats received an intracarotid 2 M urea infusion, while a second group was given a sham treatment. In both groups, the first CBF measurement was made 10 min after the intracarotid urea or sham treatment (fig. 1). The second measurement was made 3 min after an iv bolus of curare 1 mg/kg or 5 mg/kg. The third CBF measurement was made after an additional 30-min recovery period in all rats. In the final experiment, another group of rats was pretreated with cimetidine before curare injections to determine whether the H₂ receptor antagonist could inhibit increases in CBF produced by endogenous histamine release. These rats received an intracarotid injection of 2 M urea to disrupt the BBB, followed 10 min later by a first CBF measurement. They were then pretreated with cimetidine (30 mg/kg) 3 min prior to curare (1 mg/kg or 5 mg/kg) injection (fig. 1). A second CBF measurement was made 3 min after the curare injection and a third after a 30-min recovery.

**Blood-Brain Barrier Disruption**

Extravasation of Evans blue-albumin complex was used to evaluate BBB disruption in preliminary experiments. Evans blue binds with serum albumin and does not cross the BBB under normal conditions. The intracarotid urea infusion (1 ml of 2 M over 30 s) was followed 15 min later by an iv infusion of Evans blue (1 ml of 1% over 60 s) in three rats. Five minutes later, the rats were killed by an overdose of anesthetic, and the brains were removed and examined visually. The brain's surface displayed deep blue staining in both cerebral hemispheres. In contrast, rats treated with intracarotid saline showed no staining (n = 3), indicating that the BBB was intact.

**Microspheres**

Injections of microspheres were performed according to techniques previously described by Hoffman et al. Fifteen micron microspheres, labelled with cobalt-57, tin-113, or scandium-146 (New England Nuclear, Boston, MA) were used in these studies. Stock solutions containing 500,000 microspheres/ml were suspended in isotonic saline with 0.1% Tween-80. Ventricular pressure pulses were monitored before each microsphere injection. Microspheres were vortexed for 1 min, 0.2 ml withdrawn (100,000 micropheres), injected into the left ventricle via the ventricular catheter (dead space = 0.06 ml), and flushed with 0.2 ml saline. Starting immediately before each microsphere test and continuing 45 s after the end of each injection, blood was withdrawn from a femoral artery at a rate of 0.4 ml/min using a Harvard® infusion-withdrawal pump. Arterial blood samples were taken after each microsphere injection for measurement of arterial blood gas tensions and pH. Mean arterial blood pressure was measured continuously throughout the microsphere injections from the second femoral artery, to ensure that the blood pressure did not change appreciably. Heart rate was measured before each injection of microspheres. At the end of the last microsphere injection, the rat was killed by an overdose of anesthetic, and the brain was removed and sectioned into left and right cortical and subcortical samples and weighed. The activity of each isotope in samples of brain and blood was analyzed using a Nuclear Chicago 1055 Gamma Counter® and a Nuclear Data 600 multichannel analyzer. Cerebral blood flow was analyzed.
TABLE 1. Blood Pressure, Heart Rate, and Cerebral Blood Flow (CBF) Changes Produced by Histamine in Urea- and Sham-treated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Blood Pressure (mmHg)</th>
<th>Heart Rate (min⁻¹)</th>
<th>Cortical CBF (ml·100g⁻¹·min⁻¹)</th>
<th>Subcortical CBF (ml·100g⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>8</td>
<td>Control</td>
<td>159 ± 8</td>
<td>472 ± 11</td>
<td>221 ± 23</td>
<td>112 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Histamine</td>
<td>138 ± 2*</td>
<td>492 ± 9</td>
<td>219 ± 19</td>
<td>122 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery</td>
<td>155 ± 9</td>
<td>485 ± 12</td>
<td>209 ± 25</td>
<td>120 ± 11</td>
</tr>
<tr>
<td>2 M urea</td>
<td>10</td>
<td>Control</td>
<td>169 ± 5</td>
<td>486 ± 6</td>
<td>180 ± 22</td>
<td>103 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Histamine</td>
<td>140 ± 4*</td>
<td>477 ± 19</td>
<td>219 ± 50*</td>
<td>124 ± 10*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery</td>
<td>163 ± 4</td>
<td>475 ± 18</td>
<td>174 ± 22</td>
<td>122 ± 9</td>
</tr>
</tbody>
</table>

Data presented as mean ± SE. Rats in above treatment groups received each of the three tests in the order indicated. Histamine was infused IV at a rate of 10 μg·kg⁻¹·min⁻¹ for the second test in each group. * P < 0.05 histamine treatments effect (ANOVA).

according to previously reported methods. Blood samples contained 400–500 microspheres, and brain tissue samples contained 800–1000 microspheres, of each of the microsphere labels to ensure accurate measurement of tissue blood flow. Differences in right versus left cortical and subcortical CBF were statistically examined for each treatment group and, if none existed, right and left hemispheres were combined to report one tissue.

STATISTICAL METHODS

All data are reported as mean ± SE. Repeated measures analyses of variance were used to evaluate histamine and curare treatment effects in experiments 1 and 2. Differences between two groups were compared for each dose using Scheffe’s test for multiple comparisons. The level of P < 0.05 was chosen for significance for all tests.

Results

Arterial P<sub>CO2</sub> was maintained between 35 and 40 mmHg, P<sub>AO2</sub> above 100 mmHg, and pH remained at control levels under all experimental conditions in this study. There was no significant difference between treatment groups for any of these variables. No significant differences between left and right cerebral hemisphere blood flow were noted for any of the test conditions in experiments 1 or 2. Therefore, both hemispheres were combined to report cortical and subcortical tissue CBF.

Blood pressure, heart rate, and CBF changes produced by histamine infusion are shown in table 1. Histamine produced an approximate 25 mmHg decrease in blood pressure in both sham and urea pretreated rats which had stabilized for at least 3 min before microsphere tests. Histamine produced a significant increase in cortical and subcortical CBF in rats in which the BBB was disrupted by urea, but not in sham-treated animals. The effects of curare on CBF and cardiovascular parameters are shown in table 2. Both curare alone and cimetidine plus curare produced significant decreases in blood pressure in all of the treatment groups. These decreases were generally not large, and had stabilized within 1 min following the curare injection. Curare produced a significant increase in cortical CBF in rats treated with 2 M urea to disrupt the BBB, and this increase was abolished by cimetidine pretreatment. Sham-treated rats with an intact BBB showed no significant change in CBF following curare.

Discussion

Curare has previously been shown to produce hypotension as a result of ganglionic blockade and histamine release. These experiments were designed to determine whether histamine released by curare could produce cerebrovasodilation and increases in CBF, as suggested by others. In our study, neither curare injection nor direct histamine infusions increased CBF when the BBB was intact. When the BBB was disrupted by intracerebrovascular urea infusion, both histamine and curare produced significant increases in CBF. This is consistent with the results of Gross et al. that the direct cerebrovasodilating effects of histamine can be seen only after the amine is allowed access to perivascular tissue. Finally, the increases in CBF produced by curare were blocked by cimetidine pretreatment. This agrees with studies indicating that H<sub>2</sub> receptors are present in brain tissue and are primarily responsible for the cerebrovasodilatory effects of histamine.

The methods used for osmotic disruption of the BBB are a modification of a previous report by Hardebo. One ml of 2 M urea was injected into the internal carotid artery over 30 s. Because the common carotid was ligated on the same side in our study, this may have produced somewhat higher vascular concentrations of urea and higher intravascular pressure compared to the original technique. The BBB can be disrupted by either hypertensive and hyperosmotic challenges. Both of these factors may have played a role in this study. Evaluation
of the BBB with Evans blue-albumin complex 15 min after urea injection showed that both cerebral hemispheres allowed access to the dye, while sham-treated rats showed no staining of either hemisphere. This was consistent with CBF measurements, which showed that blood flow to both hemispheres increased in response to histamine and curare after urea, but not after sham treatment. We also noted a small, insignificant decrease in CBF in rats treated with urea before histamine infusion. This agrees with the results of Gross et al., who showed a similar change following urea. This may represent a short-term response to the urea or an effect of disrupting the BBB. It is apparent that cerebrovascular tissue is more subject to circulating vasoactive agents following this treatment, as demonstrated by histamine infusion.

The use of nitrous oxide as a control anesthetic may be questioned in these studies because it does not provide complete anesthesia. Although N₂O does not provide a level of anesthesia to allow surgery, it does produce analgesia without concomitant cerebral depression. It has been reported in dogs and goats that N₂O produces cerebral metabolic stimulation and increases in CBF. However, studies in rats have shown that CBF is not increased with N₂O compared to unanesthetized controls. Stress-related increases in CBF are not observed in N₂O ventilated, paralyzed rats, but are seen in the ventilating gases. These results support the use of N₂O for control measurements in the rat, and suggest that CBF may be close to that present in unanesthetized rats.

Our results agree with some, but not all, reports indicating that histamine will increase CBF only when the BBB is disrupted. Some studies have reported an increase in CBF following histamine challenges when the BBB is intact. However, these studies measured CBF either by use of a flowmeter on the common carotid or using methods that required extensive cerebral surgery. The first method does not take into account extracerebral perfusion, while the latter method may lead to a passive cerebrovascular system which does not respond in a normal manner. In this study, the increases in CBF observed with histamine and a disrupted BBB are not as large as has been reported with direct intracerebrovascular infusion. This is probably due to the peripheral vasodilating effects of histamine and resultant decreases in cerebral perfusion pressure. However, the direct cerebrovasodilating effects of histamine were apparent with intravenous infusions, as indicated by the increases in CBF.

Histamine is present in the brain and is proposed to have a neurotransmitter role. Although both classes of histamine receptors, H₁ and H₂, are present on cerebral vessels, they may mediate different responses. Wahl and Kuschinsky have shown that, for brain arterioles, H₂ receptors predominantly mediate dilatory responses to perversial histamine injections. Gross et al. also reported that pial arterioles are very sensitive to H₂ receptor stimulation and only modestly responsive to H₁ receptor-mediated drugs. We found that cimetidine, an H₂ receptor antagonist, blocked the increase in CBF produced by curare injections. The cimetidine dose, 30 mg/kg, produced

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**TABLE 2. Blood Pressure, Heart Rate, and CBF Changes Produced by D-tubocurare in Sham- and Urea-treated Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Blood Pressure (mmHg)</th>
<th>Heart Rate (min⁻¹)</th>
<th>Cortical CBF (ml·100g⁻¹·min⁻¹)</th>
<th>Subcortical CBF (ml·100g⁻¹·min⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
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<td>Control</td>
<td>152 ± 5</td>
<td>463 ± 10</td>
<td>219 ± 27</td>
<td>122 ± 10</td>
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<td></td>
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<td>1 mg/kg curare</td>
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<td>Recovery</td>
<td>155 ± 4</td>
<td>460 ± 10</td>
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<td>Sham</td>
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<td></td>
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<td>5 mg/kg curare</td>
<td>152 ± 4*</td>
<td>453 ± 15*</td>
<td>235 ± 20</td>
<td>124 ± 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery</td>
<td>166 ± 6</td>
<td>470 ± 11</td>
<td>232 ± 28</td>
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<td>2 M Urea</td>
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<td>Control</td>
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<td>478 ± 8</td>
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<td>5 mg/kg curare</td>
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<td>2 M Urea</td>
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<td>496 ± 4†</td>
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<td>110 ± 5</td>
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<tr>
<td></td>
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<td>CIM + 1 mg/kg CUR</td>
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<td>482 ± 4*</td>
<td>195 ± 8</td>
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<td>Recovery</td>
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<td>488 ± 4</td>
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<td>Control</td>
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<td>Recovery</td>
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</table>

Data presented as mean ± SE. Rats received each of the three treatments in the order indicated. CIM = cimetidine (30 mg/kg), CUR = curare.

* P < 0.05 curare treatment effect (ANOVA).
† P < 0.05 group difference compared to sham (Scheffe's test).
a 20–30 mmHg decrease in blood pressure when combined with either 1 or 5 mg/kg curare. This may have attenuated any possible histamine-induced increase in CBF if cerebral autoregulation was not intact. However, blood pressure was stable at the time of testing which should allow for normal regulation of flow. In other experiments, we have tested the effect of 30 mg/kg cimetidine alone, and have found the drug produces no change in CBF, in spite of similar decreases in blood pressure as seen here (unpublished results). This suggests that the increases in CBF produced by curare were mediated by histamine release and cerebrovascular H₂ receptor stimulation. This supports the conclusion that H₂ receptors probably mediate most of the pial arteriolar dilation when histamine is applied to cerebrovascular tissue. H₂ receptors are likely to be present in the outer pial smooth muscle layers, and are probably not very important in histaminergic dilation.

These results show that histamine or agents such as curare which release histamine can produce cerebrovasodilation and increases in CBF when the BBB is disrupted. Although curare is not used clinically to the same extent it once was, other agents, such as atracurium and morphine, also produce histamine release. Histamine release may also occur by other mechanisms, such as anaphylactoid reactions. These agents and conditions may exacerbate neurosurgical problems, such as head injury, in which the BBB is compromised and intracranial pressure elevated. Under these conditions, histamine release would lead to cerebrovasodilation, increases in cerebral blood volume, and further elevations in intracranial pressure. The use of histamine antagonists, such as cimetidine, may be appropriate before treatment with histamine-releasing drugs because of their ability to moderate the cerebrovasodilatory effects.

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References