MAMMALIAN BRAIN contains biologically significant amounts of histamine (HA) in 2 principal locations: within certain neurons, particularly those of the hypothalamus, and within mast cells, which tend to be concentrated in the leptomeninges. Neuronal HA probably functions as a neurotransmitter through a specific adenylate cyclase system that has pharmacologic properties of an \( H_3 \) receptor. The function of HA in the mast cells of the brain is unknown. Mast cells are often found in brain strategically located close to blood vessels where they may affect vascular tone and, in turn, cerebral blood flow. Schwartz has suggested that mast cell HA in the brain plays a role in inflammatory processes and vascular control, as it does elsewhere in the body.

The effect of HA on systemic vasculature has been well studied. Injected intravenously, HA constricts cardiac and pulmonary arteries and dilates the capillary bed of peripheral organs where blood pools, leading to a fall in systemic arterial pressure. Concomitantly, plasma is lost through the capillary endothelium, which accentuates shock. Injected subcutaneously, HA produces capillary hyperemia, increased vascular permeability and edema, thus mimicking, in part, the acute vascular response to injury. Although considerable information is also available about the effect of HA on cerebrovascular smooth muscle and cerebral blood flow (CBF), much of it is contradictory. The literature is not clear whether HA affects permeability of vessels of the brain as it does the permeability of vessels outside of the central nervous system.

Because of the continuing uncertainty regarding the preponderant effect of HA on CBF, the lack of information about the effect of HA on the permeability of cerebral blood vessels, and the evidence indicating biologically significant stores of HA in the brain, we undertook this in vivo study to determine the local response of the cerebral circulation of the cat to topically applied HA.

**Material and Methods**

Young cats of both sexes, weighing between 3 and 5 kg, were initially injected intramuscularly with 100 mg of ketamine and 0.2 mg of atropine sulfate. They were then intubated with a cuffed endotracheal tube, placed on a volume respirator, and paralyzed with an intravenous injection of 0.4 mg of pancuronium. Anesthesia was maintained for the remainder of the experiment with an inhaled gas mixture of 70% \( N_2O \) and 30\% \( O_2 \), supplemented with a continuous intravenous infusion of ketamine at 8 mg/kg/h. NaCl solution, 0.9\%, to which was added 1.3 mg/100 ml of pancuronium, was infused continuously through a femoral vein cutdown at 16 ml/h to maintain fluid and electrolyte balance and muscular paralysis. Rectal temperature was kept between 37°C and 38°C with an externally applied heated pad. The animal was moderately hyperventilated at a respiratory rate of 36–40 per min and a tidal volume of 50–60 cc, that served to clear inspired hydrogen, used for blood flow determinations, rapidly from the lungs. Enough \( CO_2 \) was added to the inspired gas mixture to keep \( P_C0_2 \) between 30 and 35 mm Hg. From a catheter in the femoral artery, we monitored arterial blood pressure continuously and obtained blood for gas analysis before and after each blood flow determination.

The animal was then placed prone with its head held rigidly in a standard stereotactic frame. Using microsurgical techniques to avoid traumatizing the brain, we performed bilateral cranietomies, each
measuring approximately 6 mm by 8 mm, centered 1 cm to either side of the midline and 1 cm posterior to the coronal suture. The dura was excised to expose the leptomeninges and gyri of the cerebral hemispheres. As soon as the dura was opened, irrigation of the exposed brain was begun with mock cerebrospinal fluid (CSF)* at room temperature with a pH adjusted to 7.30–7.35. The craniectomies were fashioned so that the mock CSF formed a pool about 1 mm deep over the exposed brain. An infusion pump delivered the mock CSF to each craniectomy site at a rate of 6 ml/h for the remainder of the experiment. The pools of mock CSF were heated by lamp to a temperature of approximately 35°C, as measured by a thermistor probe.

Cerebral Blood Flow Studies and Electrocorticography

Polarographic electrodes were inserted at each craniectomy site through the pool of mock CSF and leptomeninges into the crown of an exposed gyrus to a depth of 1–2 mm. The electrodes were of fine wire (0.18 mm in diameter) composed of 90% platinum and 10% iridium and insulated with Teflon. The active portion of each electrode was needle-sharp and approximately 1.5 mm long. Two electrodes were usually inserted at each site within a few millimeters of each other. The operating microscope at 40X magnification was used during the insertion in order to avoid impaling small surface vessels. After the electrodes had stabilized, local cerebral blood flow (CBF) was determined at each site by measuring clearance of hydrogen from tissue.16 Each CBF determination was begun by adding 5–10 volumes percent of hydrogen to the inspired gas mixture for 5 min, after which it was stopped abruptly. Data from the first 40 sec of washout were discarded. The following 2 min of the clearance curve, being uncontaminated by inhaled hydrogen, was considered monoexponential and analyzed as such to give CBF values as previously described.16 An average of CBF values recorded by the 2 electrodes at each site was used in analyzing the data.

Once initial baseline CBF was established, cerebrovascular reactivity was tested in each animal at the outset by determining CBF response to hypcapnia and hypercapnia. Only data from animals with normal responses were included in this report. After assessing vasoreactivity, a second baseline CBF was established and the sides were designated at random as either control or experimental. On the control side, irrigation with mock CSF was continued without interruption for the remainder of the experiment. At this point, on the experimental side, mock CSF containing histamine was substituted for plain mock CSF, all solutions having been previously adjusted to a pH of 7.30–7.35. The concentration of histamine in the initial solution varied from 10⁻⁴ M to 10⁻² M. In most experiments, 2 or more solutions of histamine were used in successively higher concentrations. At the start of each application, the site was flooded with mock CSF containing histamine so as to replace quickly the pool of fluid over the brain with the new histamine-containing solution. Then irrigation resumed at 6 ml/h. A CBF determination was made within 10–15 min of the histamine's first application. In most experiments, once the CBF measurement was completed, the site was flooded with mock CSF containing 10 times the histamine concentration of the previous solution. The process was repeated, including determination of CBF, up to a maximum histamine concentration of 10⁻² M in some experiments. HA was then washed away with fresh mock CSF, and CBF determinations were made repeatedly during the final hour of the experiment.

We did not use paraffin or mineral oil to cover the exposed cortex and prevent the hydrogen from diffusing into the air because of the necessity of suffusing the cortex with a pool of mock CSF. Earlier experiments have shown that when an animal is rapidly killed while the tissue is saturated with hydrogen, there is a similar slow hydrogen decrement, whether the electrode is deep in brain tissue in an animal with an intact skull or whether the electrode is in the superficial cortex covered with a pool of mock CSF. This slow decrement is what would be expected, theoretically, as hydrogen molecules are oxidized in the vicinity of the electrode. Because we were looking for changes in blood flow, any error introduced by hydrogen diffusion in air would be present before and after the histamine in mock CSF was applied and would also be present on the opposite, control side, also covered with mock CSF.

The same electrodes used for CBF determinations were also used in 3 experiments to record the electrocorticogram from both hemispheres after 15–20 min of irrigating one hemisphere with 10⁻⁴ M HA in mock CSF while the other hemisphere served as control. During some experiments we also photographed the brain surface through the operating microscope at 40X magnification before and during irrigation with histamine in varying concentrations.

In other experiments, the effect of the H₄ antagonist cimetidine on the response of the cerebral circulation to HA was studied. After the preparation had stabilized, a solution of mock CSF containing 10⁻⁴ M cimetidine (pH 7.30–7.35) was irrigated at 6 ml/h onto one hemisphere for one hour while the other hemisphere was irrigated with plain mock CSF. CBF determinations were made from both hemispheres as previously described. Subsequently, the irrigating solution on both sides was changed simultaneously to mock CSF containing HA 10⁻⁴ M, and during the next hour CBF determinations were repeated.

At the end of each experiment the animal was killed with a rapid intravenous injection of a saturated solution of MgSO₄. The brain was removed, sectioned, and examined for evidence of trauma or hemorrhage at the site of electrode insertion. Data from electrodes within hemorrhagic brain were discarded.

*NaCl 0.13 M; NaHCO₃ 0.026 M; dextrose 0.0030 M; KCl 0.0034 M; urea 0.0015 M; CaCl₂ 0.0014 M; MgSO₄·7H₂O 0.00041 M; NaH₂PO₄·H₂O 0.0054 M; osmolarity adjusted to 290–300 milliosmols with H₂O or NaCl.
Blood-Brain Barrier Studies

Three animals were anesthetized and prepared as previously described. In addition, both external carotid arteries were ligated and their stumps cannulated to provide a conduit to the internal carotid artery for perfusion of the brain at the end of the experiment. The animal's head was then placed in a stereotactic frame and bilateral craniectomies were performed measuring 8 mm X 16 mm, centered about 1 cm to either side of the midline and 1 cm posterior to the coronal suture, thereby exposing the dura over the crown of each middle suprasylvian gyrus. The dura was excised and irrigation of the gyri began with mock CSF. At this point, 0.2-0.5 millicurie of $^{125}$I-albumin was given intravenously. One hemisphere was then designated as control and irrigated for 1 h with mock CSF at 6 ml/h. The other experimental hemisphere in 2 animals was irrigated for 1 h with mock CSF containing $10^{-1}$ M HA (pH 7.30-7.35) at 6 ml/h. Halfway through the irrigation blood was drawn for later determination of the level of radioactivity in the serum. After an hour of irrigation, the animal was heparinized and then killed with an intravenous injection of a saturated solution of MgSO$_4$. Both previously exposed common carotid arteries were quickly ligated, after which both internal carotid arteries were simultaneously perfused, via the previously placed cannulae, with 50 ml of warm saline followed by 50 ml of buffered formalin in order to wash the cerebral vascular bed free of blood and partially fix the brain to make it easier to handle. The portion of each middle suprasylvian gyrus that had been exposed to either HA or mock CSF was removed en bloc. After the leptomeninges were peeled off, the block of brain was chilled in liquid nitrogen until firm and sectioned on a plane parallel to the surface of the crown of the gyrus into slices 1 mm thick. Each slice was weighed in a tared vial and its $^{125}$I activity measured in a well-type scintillation counter. $^{125}$I activity of serum was determined simultaneously. The albumin space of each slice was computed from the following formula:

$$\text{Albumin space} = \frac{^{125}\text{I}}{^{125}\text{I}_{\text{activity/gm brain}}/^{125}\text{I}_{\text{activity/gm serum}}} \times 100.$$ 

To serve as a positive control, a third experiment was performed in which a solution containing 6.2 M urea, that is known to open the blood-brain barrier to albumin, was substituted for $10^{-1}$ M HA.

Diffusion Studies

The extent to which HA penetrates the brain after 1 h of epiarachnoid irrigation was determined in 2 animals. After the brain was exposed through bilateral craniectomies, as described in the previous section, one hemisphere was irrigated for one h with mock CSF containing $10^{-1}$ M tritium-tagged HA. The other hemisphere served as a control. At the end of the irrigation period, the brain was blotted dry with filter paper, the animal killed with an intravenous injection of a saturated solution of MgSO$_4$, the brain was removed within 30 sec and immersed in liquid nitrogen until firm. After the leptomeninges were removed, a wedge-shaped segment was cut from that portion of the gyrus that had been irrigated, with the base of the wedge being formed by the subpial crown of the gyrus and the apex formed by the subcortical white matter. The wedge-shaped segment of brain was then sliced at 1 mm intervals on a plane parallel to the base. Each slice was placed in a tared vial and its $^{3}H$ activity measured in a liquid scintillation counter. Samples of tissue from the control side as

![Figure 1. Focal CBF response to HA in 17 experiments. Each point represents a separate CBF determination. Line joins data points from a single experiment. Statistical analyses for data in this graph are given in table.](https://stroke.ahajournals.org/)
FIGURE 2. Photographs of the surface of cat cerebrum made during a single experiment before (A) and during (B-D) irrigation with increasingly concentrated solutions of HA in mock CSF. HA dilates all surface vessels, arteries (open arrows) more than veins (closed arrows). A) Before HA; CBF = 55 ml/100 gm/min; B) during 10^−4 M HA; CBF = 63 ml/100 gm/min; C) during 10^−4 M HA; CBF = 82 ml/100 gm/min; D) during 10^−4 M HA; CBF = 116 ml/100 gm/min.

well as aliquots of irrigation fluid were similarly processed. The amount of exogenous HA (or its metabolites) in each slice of brain, expressed as a percentage of the concentration of HA in irrigation fluid, was calculated as follows:

Exogenous HA in brain =
\[ \frac{3H \text{ activity/gm brain}}{3H \text{ activity/gm irrigation fluid}} \times 100. \]

**Results**

CBF

Valid CBF data were obtained from 17 cats: 4 successively higher concentrations of histamine were used in 4 animals; 3 concentrations of histamine were used in 3 animals; 2 concentrations were used in 4 animals, and in the remaining animals only one concentration of histamine was used (fig. 1).

Throughout each experiment, which at times lasted up to 8 h, mean arterial blood pressure, rectal temperature, and blood gases remained stable and normal. Mean arterial blood pressure ranged between 130 and 195 mm Hg, rectal temperature between 37°C and 38°C, Pco2 between 30 and 36 mm Hg, pH between 7.20 and 7.36, and Po2 between 125 and 170 mm Hg.

Focal irrigation of a cerebral hemisphere with histamine produced no discernible systemic effect. Mean local CBF values on the control side were not significantly different from baseline CBF values on the experimental side, and they did not vary significantly throughout each experiment. Within 10–15 min of the application of histamine, CBF increased in a dose-dependent manner. The higher concentrations about doubled the average CBF (table and fig. 1). Upon cessation of the application of histamine, CBF usually returned to baseline levels within 30 to 60 min.
Photographs of the irrigated surface showed that surface arteries and, to a lesser extent, veins dilated in a dose-dependent fashion when exposed to histamine (fig. 2).

The effect of H₂ blockade with 10⁻¹ M cimetidine on HA-induced hyperemia was determined in 4 animals. After irrigation with 10⁻⁸ M HA, mean CBF of untreated hemispheres increased 137%, whereas, mean CBF of hemisphere previously irrigated with 10⁻² M cimetidine increased only 6% (fig. 3).

Electrocorticogram

HA 10⁻⁴ had no effect on the electrocorticogram recorded from electrodes that were also used to determine CBF (fig. 4).

Blood-Brain Barrier

HA had little effect on the blood-brain barrier to albumin, as demonstrated by failure of 10⁻¹ M HA, applied epiarachnoid for 1 h, to increase appreciably the albumin space of the brain. In contrast, a 6.2 M urea solution used in the same way resulted in a marked increase in the albumin space (fig. 5).

Diffusion of HA

After 1 h of epiarachnoid irrigation, the mean concentration of exogenous HA (and metabolites) in the first 1 mm of brain subjacent to the pool of irrigating solution was approximately 15% of the concentration of HA in the irrigation fluid. The concentration of HA in tissue samples decreased by approximately one-half for each additional 0.4 mm that the samples were located from the leptomeningeal surface (fig. 6).

Discussion

When applied topically to the brain so as to bypass the blood-brain barrier, HA quickly overrode autoregulatory mechanisms that normally control CBF and consistently produced a dose-related reversible local hyperemia in an otherwise stable and intact cerebral circulation. In the absence of any concurrent effect on the electrocorticogram recorded from the same electrodes used to determine CBF (fig. 4), we have assumed that HA acts directly on cerebrovascular smooth muscle rather than indirectly through an accumulation of acid metabolites from increased neuronal activity. The hyperemia appears to be a specific action of HA that is mediated through H₂ receptors since it was blocked by the H₂-antagonist cimetidine. These findings are in harmony with some
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Before Histamine

After Histamine

FIGURE 4. Electroencephalogram recorded from the exposed cerebrum of the cat before (left) and after (right) irrigating the brain surrounding the electrode in the right hemisphere with 10^{-3} M HA in mock CSF for 17 min. In the left hemisphere (control) HA had no discernible effect on the recorded electrical activity.

Experiments performed in vitro on isolated muscle-strip preparations. Although the significance of these observations remains to be defined, they imply that HA is pharmacologically capable of playing a role in regulating the response of the cerebral microcirculation to a variety of physiologic and pathologic stimuli.

The hyperemia that characterizes HA's action on the cerebrovascular bed could not have been predicted with confidence from the available literature because there is lack of agreement among results from various investigators. Sokoloff and others concluded that HA is a potent dilator of cerebral vessels. The study of Edvinsson and Owman indicated that the results obtained in vitro depend on conditions under which HA is tested. They found that when smooth muscle strips obtained from the cerebral arteries of human subjects and cats are bathed by an H_2 antagonist to prevent any contractile effect, and when they are tonically contracted with serotonin, HA will produce a dilatory response mediated by a specific H_2 receptor. They also found that in the presence of the H_2 antagonist burimamide, HA induced a dose-dependent contraction of the smooth muscle via a nonspecific action. Because of the contrived experimental conditions, it would be difficult to predict from the in vitro experiments of Edvinsson and Owman which effect, if any, would predominate in vivo. Indeed, in the in vivo experiments of Rosenblum, HA applied topically to pial arteries of mice had no effect. However, later in vivo studies by Kuschinsky and Wahl demonstrated that HA applied topically in microliter quantities dilated the pial arteries of the cat. They also showed that the dilatation could be blocked by cimetidine. Our results corroborate those of Kuschinsky and Wahl and provide additional evidence indicating that the preponderant functional effect of HA on the intact mammalian cerebral circulation is not only to dilate the pial vessels but to increase CBF as well. Simultaneous determination of blood vessel diameter (fig. 2) and local CBF (fig. 1) permitted anatomic and physiologic data to be correlated one with the other. Topical application of HA avoided confusing systemic effects, bypassed the blood-brain barrier to allow access to vascular receptor sites, and permitted each animal to serve as its own control.

One may question whether the concentrations used in these experiments to flood the tissue with HA are physiologically relevant. Some information suggests that they are comparable to concentrations
The hypothesis that HA may participate in the acute response of the brain to injury and other inflammatory stimuli is not new. A previous report from this laboratory implicated HA in the acute hyperemic response of the central nervous system to injury. It was shown that H₁ and H₂ antagonists, administered systemically together, prevented hyperemia that develops in tissue adjacent to an injured segment of spinal cord. Clasen et al. tried unsuccessfully to reduce the amount of edema produced by a standardized cryogenically induced cerebral injury with a combination of a glucocorticosteroid and the H₁ antagonist chlorpheniramine. Outside of the central nervous system, HA increases vascular permeability that leads to plasma exudation and the accumulation of interstitial edema. This effect on endothelium of small vessels is apparently distinct from its action on smooth muscle. It has been unclear as to whether HA can open the blood-brain barrier that is formed by the tight junctions between endothelial cells of the small vessels of the brain. Our investigation of the effect of HA on the albumin space of the brain suggests that it has little effect on the permeability of the blood-brain barrier to protein. Since a $10^{-4}$ M solution of HA was used in the permeability experiments to irrigate the brain, we estimate, from our diffusion studies, that a mean concentration of more than $10^{-2}$ M HA was attained in the first 1 mm of brain. Nevertheless, the albumin space was not appreciably increased despite the high concentration of HA.

To summarize, HA readily passes through the leptomeninges after epiarachnoid application and rapidly produces a predictable, reversible, dose-related dilation of cerebral blood vessels that is accompanied by increased CBF. Vasodilatation and secondary hyperemia is a specific effect of HA mediated through H₂ receptors that can be blocked by cimetidine and which results from a direct effect on cerebrovascular smooth muscle and is not dependent on increasing neuronal activity. Finally, HA has little effect on cerebrovascular permeability to protein. We have concluded that HA is pharmacologically capable of participating directly in the acute hyperemic response of the cerebral microcirculation to injury but it probably accounts for little or none of the accompanying vasogenic edema.

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