Altered response to histamine in brain tumor vessels: the selective increase of regional cerebral blood flow in transplanted rat brain tumor

TOMOJIROU NOMURA, M.D., KIVONOBU IKEZAKI, M.D., YOSHIHIRO NATORI, M.D., AND MASASHI FUKUI, M.D.

Department of Neurosurgery, Neurological Institute, Kyushu University, Faculty of Medicine, Fukuoka, Japan

The authors studied the effect of intracarotid administration of histamine on the regional cerebral blood flow (rCBF) in transplanted rat C6 glioma by the hydrogen clearance method. Histamine infusion at doses of 1 and 10 μg/kg/min produced an increase of rCBF in the tumor (24.6% ± 16.4%, p < 0.002, and 37.6% ± 18.2%, p < 0.0001, respectively) and also in brain surrounding the tumor (26.8% ± 16.2%, p < 0.002, and 34.9% ± 9.2%, p < 0.0001, respectively) without any significant changes in the ipsilateral hemisphere. Intravenous administration of pyrilamine (H1 antagonist) and cimetidine (H2 antagonist) reduced blood flow responses to histamine: cimetidine was a more effective blocking agent than pyrilamine. Intracarotid infusion of histamine (1 and 10 μg/kg/min) with intravenous injection of Evans blue dye disclosed the selective extravasation of dye in the tumor and the brain surrounding the tumor.

These results indicated that brain tumor vessels could respond to histamine differently than normal brain capillaries. The mechanism of selective response to histamine could be explained either by increased permeability or by altered characteristics of histamine receptors in the tumor vessels.

KEY WORDS: histamine • regional cerebral blood flow • brain neoplasm • C6 glioma • drug delivery • neurotransmitter • rat

VARIOUS neurotransmitters, such as noradrenaline, 5-hydroxytryptamine, acetylcholine, and neuropeptide Y, have been reported to regulate regional cerebral blood circulation and permeability.10,46 Their action on in vitro cerebral vessels, however, have differed according to drug concentration, the diameter of vessels, vascular tonus, and the condition of the endothelium. On the other hand, blood vessels in brain tumors are structurally17,28,29 and biochemically,3,26,27,33 different from normal cerebral vessels. The regulatory action of neurotransmitters to brain tumor vessels has been studied only with noradrenaline. Panther, et al.,33 reported a reduction of intratumoral blood flow following the intravenous infusion of noradrenaline in a canine brain-tumor model.

Histamine is regarded as one of the most potent natural vasoactive substances in the peripheral organs,31,55 and has been detected in cerebral perivascular mast cells28,29 and the cerebrovascular smooth muscle.13 Thus, this amine has been proposed as a vasoactive neurotransmitter14,38 because of the histaminergic innervation of the cerebral microvasculature.49 Histaminergic vasodilatory effects on the normal pial vessels and arterioles have been shown in vitro in isolated arteries7,10,39,45 and in vivo with perivascular microapplication5,9,17,45 and cortical superfusion.23,37 The vasodilatory effect of histamine is mediated by H1 and H2 receptors.3,26,28,31,55,59-11,15,17,19,43,45 There are, however, few studies of histamine in terms of the regional cerebral blood flow (rCBF) in the normal cerebral vessels. A histamine-induced increase of the rCBF in vivo has been obtained only following a hyperosmolar disruption of the blood-brain barrier,17 and these changes have not yet been studied in brain tumors.

In this study, we analyzed in rats the effect of intracarotid administration of histamine on the rCBF in brain tumors as well as in brain tissue surrounding the tumor (BST) in the ipsilateral hemisphere. In addition, the mechanism of histamine-induced selective rCBF increase in the tumor is discussed with the results of a receptor antagonist study and an Evans blue dye (EB) extravasation test.
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Materials and Methods

Forty-five male Wistar rats, each weighing 200 to 260 gm, were used for the experiment. Four rats underwent sham operations and the remainder received tumor cell implantation.

Tumor Inoculation

First, C6 glioma cells were subcultured in Ham's F-10 medium containing 10% fetal bovine serum.22 Tumor cells (5 × 10^6 in a 5-μl solution of Dulbecco's modified Eagle's medium with methyl cellulose) were stereotactically implanted into the right cerebral hemisphere of rats while anesthetized with intraperitoneal pentobarbital sodium (Nembutal, 50 mg/kg). The sham operation was performed with an injection of the same solution without tumor cells.

Animal Preparation

The rats were studied under intraperitoneal pentobarbital anesthesia (50 mg/kg) 11 to 21 days after tumor inoculation. A tracheotomy was performed with catheter placement in the femoral arteries, veins, and internal carotid artery, as previously described in detail.2 Each animal was mounted onto a stereotactic apparatus, immobilized with intravenous pancuronium (0.6 mg/kg), and mechanically ventilated with room air. During the experiment, anesthesia was maintained with 0.2% to 0.5% halothane, and the arterial blood pressure was monitored. End-tidal CO₂ and rectal temperature were also monitored continuously and adjusted regularly throughout each experiment.2 After the arterial blood pressure stabilized, arterial blood samples were taken for blood gas analysis.

Blood Flow Measurement

Two burr holes were made in the parietal bone; one was placed 4 mm lateral and 2 mm anterior to the bregma in both the sham-operated rats and the tumor-implanted rats, and the other was placed 5 mm posterior to it in the sham-operated rats and 3 to 8 mm posterior in the tumor-implanted rats. Two Teflon-coated platinum electrodes, 0.2 mm in diameter, were inserted through the burr holes to measure the rCBF. The referred electrodes were then inserted under the skin. The rCBF was measured by the hydrogen clearance method with a digital tissue blood flowmeter,* as described previously.2

Experimental Protocol

In the control experiments, all animals were given 0.9% saline through the internal carotid artery by a constant infusion at a rate of 1.5 ml/hr. Histamine was dissolved in 0.9% saline and administered into the carotid artery at the same infusion rate as saline.

Histamine infusion was begun 10 minutes before blood flow measurements, at doses of 1, 10, and 20 μg/kg/min in the sham-operated rats, and 1 and 10 μg/kg/min in the tumor-implanted rats. Histamine receptor blocking experiments were conducted by the method of Gross, et al.19 The histamine H₂ receptor antagonist pyrilamine and the histamine H₂ receptor antagonist cimetidine were administered intravenously (5 mg/kg) 10 minutes before histamine infusion. After the blood pressure stabilized, rCBF was measured during administration of histamine.

To assess blood-brain barrier permeability, histamine was administered into the carotid artery at a dose of 1 or 10 μg/kg/min for 10 minutes in five rats for each dose. A 2-ml/kg bolus of 2% EB was given intravenously immediately after histamine infusion. Saline was infused instead of histamine in five control rats. The rats were decapitated 20 minutes after the EB injection.

Histological Studies

After completion of the experiments, the rats were killed with an overdose of pentobarbital sodium, and the brains were removed and fixed with 10% formalin for blood flow measurement. The rats receiving EB injection were perfused with Ringer's solution through the aorta until the fluid from the caval vein became clear. Extravasation of EB was visually investigated in the removed brain and graded as none, slight, or moderate. The specimens were then embedded in paraffin or frozen, then sectioned, stained with hematoxylin and eosin, and examined microscopically. The electrode tracks were carefully examined, and the brains with either massive hemorrhage or necrosis were excluded from analysis. The ipsilateral basal ganglion 5 mm from the needle track in the sham-operated rats was defined as the normal control. Portions of the brain within 3 mm of the tumor edge were defined as the BST, while portions of the brain more than 3 mm from the tumor edge were defined as the ipsilateral hemisphere without tumor cells after histological confirmation.

Statistical Analysis

The data were expressed as mean ± standard deviation. Statistical analysis was performed with the paired and unpaired Student's t-test, and analysis of variance. A probability of 0.05 or less was considered significant.

Results

Physiological Status

The animals were separated into two groups: the sham-operated group and the tumor-implanted group. Before drug administration, arterial blood gas (pH, PaCO₂, and PaO₂) measurements and the mean arterial pressure were obtained (Table 1). There were no significant differences in these physiological parameters between the two groups.

Regional Cerebral Blood Flow

Because the intracarotid infusion of histamine at a dose of 20 μg/kg/min significantly decreased the systemic blood pressure in the sham-operated rats (116.3 ± 4.8 to 95.0 ± 4.1 ml/min/100 gm, p < 0.004), further experiments were performed with histamine at doses of 1 and 10 μg/kg/min.

* Digital UH-meter, Model MHG-D1, manufactured by Unique Medical Co., Tokyo, Japan.
Table 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sham-Operated Rats</th>
<th>Tumor-Implanted Rats</th>
</tr>
</thead>
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<tr>
<td>mean arterial pressure</td>
<td>121.3 ± 4.8</td>
<td>117.5 ± 5.4</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>82.6 ± 6.4</td>
<td>81.4 ± 10.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.1</td>
<td>7.4 ± 0.1</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>32.8 ± 1.6</td>
<td>32.2 ± 1.7</td>
</tr>
<tr>
<td>end-tidal CO₂</td>
<td>36.0 ± 0.2</td>
<td>35.8 ± 0.3</td>
</tr>
<tr>
<td>no. of rats</td>
<td>4</td>
<td>30</td>
</tr>
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</table>

* Values are presented as mean ± standard deviation. Values were compared for both groups using an unpaired t-test, and the differences were not significant.

Sham-Operated Rats. The baseline rCBF in the sham-operated lesion was significantly higher than that of the normal control (59.2 ± 13.9 and 39.9 ± 6.1 ml/min/100 gm, respectively, p < 0.05). Although the experiments were performed 14 days (on the average) after the sham operation, some tissue injury due to needle insertion might have been responsible for such a difference. The intracarotid infusion of histamine, however, did not significantly change rCBF either in the sham-operated lesion or in the normal control (Table 2).

Tumor-Implanted Rats. Before histamine infusion, the mean rCBF for both infusion rates calculated together was significantly higher than that in the ipsilateral hemisphere (63.4 ± 14.5 and 35.4 ± 9.6 ml/min/100 gm, respectively, p < 0.0002). The rCBF in the BST was not significantly different from that in the ipsilateral hemisphere (37.3 ± 9.0 and 35.4 ± 9.6, respectively, p = 0.61). Intracarotid infusion of histamine significantly increased the rCBF both in the tumor and the BST (p < 0.002) (Table 2). In contrast, the rCBF in the ipsilateral hemisphere did not increase during intracarotid injection of histamine, even at a dose of 10 μg/kg/min.

The percent increases in the rCBF produced by 1 and 10 μg/kg/min of histamine were 24.6% ± 16.4% and 37.6% ± 18.2% in the tumor, respectively; in the BST, levels of 26.8% ± 16.2% and 34.9% ± 9.2% above preinfusion values were observed, respectively. These percent increases of the rCBF in the tumor and the BST were significantly higher than that in the normal or sham-operated rats (p < 0.02) (Table 2).

The specific histamine receptor antagonists, pyrilamine (H₁) and cimetidine (H₂), did not affect either the rCBF or the mean arterial pressure at a dose of 5 mg/kg intravenously (data not shown). When the rats were pretreated with histamine blockers 10 minutes before histamine infusion, the percent increases of the rCBF were 14.3% ± 1.8% in the tumor and 23.1% ± 5.1% in the BST with pyrilamine infusion, and 10.9% ± 4.1% in the tumor and 9.2% ± 2.7% in the BST with cimetidine infusion (Fig. 1). The histamine-induced selective increase of the rCBF in the tumor and the BST was significantly reduced with preadministration of the H₁ blocker by 63% and 34% (p < 0.05), and with the H₂ blocker by 71% and 74% (p < 0.01), respectively. The H₂ antagonist was more potent in reducing the histamine-induced rCBF increase both in the tumor and in the BST when compared to the H₁ antagonist, although there was no significant difference between them.

Blood-Brain Barrier Permeability

All five control rats tested for blood-brain barrier permeability showed no definite extravasation of EB

Table 2

<table>
<thead>
<tr>
<th>Histamine Infusion Rate (μg/kg/min)</th>
<th>Rat Group &amp; Region Studied</th>
<th>rCBF (ml/min/100 gm) Preinfusion Postinfusion</th>
<th>p Value†</th>
<th>Changes of rCBF</th>
<th>p Value§</th>
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<tbody>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td>Percent Change</td>
<td></td>
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<tr>
<td>1</td>
<td>normal</td>
<td>39.9 ± 6.1</td>
<td>41.2 ± 6.0</td>
<td>NS</td>
<td>3.6 ± 10.6</td>
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<td></td>
<td>sham</td>
<td>59.2 ± 13.9</td>
<td>62.4 ± 13.9</td>
<td>NS</td>
<td>5.8 ± 7.0</td>
</tr>
<tr>
<td>10</td>
<td>normal</td>
<td>39.9 ± 6.1</td>
<td>46.4 ± 2.2</td>
<td>NS</td>
<td>12.0 ± 13.3</td>
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<tr>
<td></td>
<td>sham</td>
<td>59.2 ± 13.9</td>
<td>65.9 ± 18.5</td>
<td>NS</td>
<td>11.2 ± 13.0</td>
</tr>
<tr>
<td>tumor-implanted rats</td>
<td></td>
<td></td>
<td></td>
<td>Percent Change</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ipsi</td>
<td>35.9 ± 7.0</td>
<td>39.3 ± 7.0</td>
<td>NS</td>
<td>10.0 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>BST</td>
<td>37.1 ± 8.8</td>
<td>46.7 ± 11.5</td>
<td>&lt;0.002</td>
<td>26.8 ± 16.2</td>
</tr>
<tr>
<td>10</td>
<td>tumor</td>
<td>65.6 ± 18.8</td>
<td>81.8 ± 30.2</td>
<td>&lt;0.002</td>
<td>24.6 ± 16.4</td>
</tr>
<tr>
<td></td>
<td>ipsi</td>
<td>35.0 ± 12.3</td>
<td>40.5 ± 15.1</td>
<td>NS</td>
<td>14.3 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>BST</td>
<td>37.5 ± 9.2</td>
<td>50.7 ± 11.6</td>
<td>&lt;0.0001</td>
<td>34.9 ± 9.2</td>
</tr>
<tr>
<td>1</td>
<td>tumor</td>
<td>61.2 ± 10.2</td>
<td>84.1 ± 18.3</td>
<td>&lt;0.0001</td>
<td>37.6 ± 18.2</td>
</tr>
</tbody>
</table>

* Values are presented as means ± standard deviation. Normal = ipsilateral hemisphere 5 mm posterior to the sham-operated lesion; sham = sham-operated lesion; ipsi = ipsilateral hemisphere more than 3 mm posterior to the tumor edge; BST = brain surrounding the tumor; NS = not significant.
† Preinfusion vs. postinfusion values of rCBF at each region using paired t-test.
§ Significance of difference from percent changes of normal rCBF at each dose using analysis of variance.
‡ Preinfusion vs. postinfusion values.
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macroscopically after the intracarotid saline infusion (Fig. 2). Histamine at a dose of 1 μg/kg/min in five rats produced a slight homogeneous EB extravasation in the tumor except for one which showed no definite extravasation of EB and no significant extravasation in the extratumoral brain. In the five rats receiving histamine at a dose of 10 μg/kg/min, a moderate homogeneous extravasation of EB both in the tumor and in the BST was observed in all five rats without significant extravasation in the extratumoral ipsilateral brain.

Discussion

There were three principal findings in this study. 1) The intracarotid infusion of histamine increased rCBF both in the tumor and in the BST without affecting rCBF in the extratumoral ipsilateral cerebrum. 2) The histamine-induced selective increase of the rCBF in the tumor and the BST was blocked by intravenous administration of histamine H1 and H2 receptor antagonists. 3) Intracarotid histamine infusion obtained a selective increase in blood-tumor barrier permeability.

Histamine has been reported to increase blood flow in the cerebral arterial trunks in dogs; for example,

FIG. 1. Graph showing the percent changes of regional cerebral blood flow (rCBF) in the tumor and in the brain surrounding the tumor (BST) after intracarotid infusion of histamine at a dose of 10 μg/kg/min with or without pretreatment of either pyrilamine (H1 antagonist) or cimetidine (H2 antagonist) at a dose of 5 mg/kg. P values = compared with no blockade, using analysis of variance. The numbers in parentheses represent the number of rats studied at each region.

FIG. 2. Histological specimens stained with hematoxylin and eosin (H & E, upper) or Evans blue dye (EB, lower) after infusion of saline (left) or histamine (1 μg/kg/min, center, or 10 μg/kg/min, right). A natural contrast of tissues can distinguish the tumor from the normal brain. Macroscopically, however, there was no definite EB extravasation either in the normal or tumor tissue after intracarotid saline infusion (lower left). A slight homogeneous EB staining (arrows) in the tumor was noted after intracarotid histamine infusion at a dose of 1 μg/kg/min (lower center). Histamine infusion at a dose of 10 μg/kg/min produced moderate EB staining (arrowheads, lower right) both in the tumor and in the brain surrounding the tumor.
basilar arterial blood flow was increased by 145% (25 
μg/kg, intravenous injection) and carotid arterial blood
flow by 400% (6.4 μg/kg, intra-arterial injection) by
electromagnetic flow measurement. On the other hand,
rcBF in the brain parenchyma, measured by the 133Xe
clearance method, was not affected by intracarotid
administration of histamine in a range of 0.1 to 60 μg/
kg/min.5,12,14 Our findings, which showed no significant
changes of the rCBF in the normal brain, also support
this phenomenon. There was an interesting report by
Gross, et al.,18 showing that intracarotid administration
of histamine (6 to 60 μg/kg/min) increased the rCBF
in the normal brain only after the osmotic opening of
the blood-brain barrier in anesthetized rats by 27% to
50% using the 133Xe clearance method of measurement
and by 9% to 31% based on 14C-iodoantipyrine auto-
radiography. In our study, intracarotid infusion of his-
tamine (1 or 10 μg/kg/min) also increased the rCBF
both in the tumor and in the BST by 25% or 38%,
respectively, without affecting the rCBF in the extratu-
moral ipsilateral cerebrum. Considering that increased
permeability was found in the tumor and/or the BST
in the experimental brain tumors,2,3,4,10,17,49 these find-
ings lead us to the following speculations. 1) In normal
conditions, intratumoral histamine increases the blood
flow in the cerebral main arterial trunk and does not
affect the capillary blood flow (rcBF) because of the
existence of the blood-brain barrier and autoregulation.
2) The osmotic disruption of the blood-brain barrier
and/or increased permeability in the brain tumor might
allow intraluminal histamine to extravasate into the
luminal side at the level of the arterioles. 3) Thereafter,
histamine binding to H1 and H2 receptors, which exist
in the inner layers of vascular smooth muscle, produce
their vasodilating effects selectively on capillaries in the
affected lesion.

Intracarotid administration of histamine induced the
selective increase of EB extravasation in the brain tu-
mor and the BST in our study. Although there have
been reports that the cortical superfusion (10−7 to 10−4
M)3,13 or intravascular administration (2 to 500 μg/kg/
min)18,19 of histamine elicited extravasation of various
tracers into the normal brain tissue, a selective increase
of blood-tumor barrier permeability was obtained in
our experimental protocol. Thus, the histamine-in-
duced selective increase of the rCBF in the tumor and
the BST might be enhanced by the increased permea-

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d
bility conducted by histamine itself.

The intratumoral blood flow shows an altered re-
sponse to vasoactive agents,2,3,6,18 induced hyperten-
sion,4,25,44 and CO2 changes31,32 when compared to the
blood flow in the normal brain tissue. In addition to
the disruption of the blood-brain barrier in tumor
tissue, the altered reactivity to vasoactive agents and
the loss of autoregulation in the tumor may participate
in these phenomena. Natori and colleagues26,27 sug-
gested that the altered reaction of tumor vessels to
adenosine triphosphate and adenosine was due to the
hyperreaction of the receptor site in the endothelium
and smooth muscle. Although two classes of histamine
receptor antagonist could not block the histamine-in-
duced rCBF changes completely, our receptor blocking
study might indicate that the rCBF in tumor vessels
was also controlled by histamine and its receptor in-

Because of the different results among various animal
species, it has not been clarified yet how these subclasses
of histamine receptor interact with each other for brain
circulation. Using isolated rabbit cerebral arteries, Ser-
combe, et al.39 showed that the H1 receptor in smooth
muscle mediates endothelium nondependent vasoco-
striction and the H2 receptor in endothelium causes
endothelium-dependent vasodilation. Recently, several
reports have indicated that histamine-induced vasodi-
latation was mediated by both the H1 receptor on en-
dotheium, which released endothelium-derived relax-
ing factor, and the H2 receptor in the smooth muscle.4,43
In the present study, the H2 antagonist was a more
potent inhibitor, indicating that the histamine-in-
duced rCBF increase in the tumor was more depend-
ent on the H2 receptor-mediated response. In addition,
it is not clear whether the receptors, which account
for histamine-induced rCBF changes, exist in the en-
dotheium or in the smooth muscle, or whether tumor
and the BST vessels might, in fact, possess different
receptor characteristics in addition to morphological
changes.20,28,29

The selective action of histamine on the rCBF and
permeability of brain-tumor vessels is thus considered
to be an interesting phenomenon. Using the altered
action of histamine as a means of selectively increasing
permeability and rCBF in the tumor tissue may con-
tribute to the selective delivery of the water- and lipid-
soluble therapeutic agents. It is hoped that a clarifica-
tion of the mechanisms of action of vasoactive agents
on the blood vessels of the normal brain and tumor
may assist in the management of malignant brain tu-
mors either by chemotherapy or by other supplemen-
tary therapies.

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Address reprint requests to: Tomojirou Nomura, M.D., Department of Neurosurgery, Neurological Institute, Kyushu University, Faculty of Medicine 60, Fukuoka 812, Japan.