Partial reversal of asymmetry in microvessel neurochemical changes after ischemia by corpus callosum section

M. S. Magnoni, Ph.D., H. Kobayashi, Ph.D., S. Govoni, Ph.D., F. Battaini, Ph.D., G. Pasinetti, Ph.D., and M. Trabucchi, M.D.

ABSTRACT Common carotid occlusion in the rat significantly decreases the density of $\beta$-adrenergic receptors in preparations of microvessels obtained from ipsilateral and contralateral cerebral cortices. The disruption of nerve pathways connecting the hemispheres (callosal transection) partially reverses the effect of common carotid occlusion on $\beta$-adrenergic receptor density in capillaries of the contralateral cortex. In addition, the destruction of the central noradrenergic system by intraventricular injection of 6-hydroxydopamine abolishes the effect of ischemia on capillary $\beta$-adrenergic receptor function in both hemispheres. The results suggest that $\beta$-adrenergic receptors located on microvessels are partially regulated by neuronal pathways and that focal ischemia induces neurochemical and functional changes in remote areas of the brain.

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THE BRAIN is extremely sensitive to ischemia; in fact the deprivation of blood supply induces a failure in synaptic transmission and neuronal death. Both neurotransmitter function and metabolism appear altered in various cerebral areas after ischemia.1-12 These events are accompanied by changes in microvascular functions such as increased permeability to water and decreased glucose uptake and oxygen consumption.13-16 Recent publications have demonstrated the existence of $\beta$-adrenergic receptors coupled to adenylate cyclase in cerebral capillaries, which seem to be involved in the regulation of microvascular function.17-24 In a previous study we investigated the effect of common carotid occlusion on $\beta$-adrenergic receptor function in preparations of cerebral capillaries obtained from gerbils and rats.25, 26 The results indicated a significant decrease in the number of $\beta$-adrenergic receptors in brain microvessels of both the ipsilateral and contralateral hemispheres. Notably, in occlusion of either the right or left carotid artery the reduction in receptor density is more pronounced in the left hemisphere, suggesting a peculiar sensitivity of the left side of the brain to the effects of ischemia.25, 26 The loss of binding sites may partially be due to the massive release of catecholamines from ischemic neurons into the synaptic cleft.3, 4 On the other hand, this event does not explain the major decrease in receptor number in cerebral capillaries of the contralateral cortex, particularly in the case of right carotid ligation.

It is known that local cerebral ischemia may cause biochemical and functional alterations in areas of the brain distant from the site of the injury.10, 27-31 To investigate the role of neuronal pathways connecting the hemispheres in the biochemical changes observed in the contralateral side of the brain, $\beta$-adrenergic receptors were measured in brain capillaries of right or left carotid-ligated rats in two experimental conditions: after the section of corpus callosum and after selective lesion of the central noradrenergic system by intraventricular injection of 6-hydroxydopamine (6-OHDA).32

Materials and methods

Adult male Sprague-Dawley rats of about 150 to 170 g were used. Each experiment was performed with 20 animals per group; the data presented are means of four different experiments.

Corpus callosum section. Rats were placed in a stereotaxic apparatus under chloral hydrate anesthesia (400 mg/kg). A midline incision was made through the scalp and underlying membranes scraped away from the skull. A rectangle of bone ap-
proximately 10 mm long and 6 mm wide centered on the midline suture was removed, leaving the dura intact. The corpus callosum was sectioned with a thin blade fixed to the stereotaxic apparatus. A preliminary study indicated the coordinates required for the lesion: the length was from 5 mm posterior to bregma to 3 mm anterior to bregma (−5 to +3); the depth from the dura was 3.5 mm from −5 to +0.5 mm and 4.5 from +0.5 to +3 mm.

The sagittal sinus was gently displaced while the blade was lowered along the midline and was then left to return elastically to its original position.

The mortality rate (due principally to lesion of the sagittal sinus) was about 6%. Animals were injected with chloramphenicol (80 mg/kg im) immediately after the procedure and on the following day. After 2 weeks of recovery, the rats underwent right or left carotid occlusion.

After dissection from its accompanying vagus nerve and vein, the carotid was rapidly double ligated with 4-0 silk suture. In sham-operated controls the vessel was exposed and dissected from the vagus nerve but not ligated. In both sham-operated and carotid-ligated rats, the manipulation of the artery may have caused damage to the sympathetic chain (manifested by the presence of ptosis in a small percentage of animals).

The occlusion of a common carotid in the rat does not produce clinical signs of cerebral infarction, since blood supply to the brain is also provided by basilar circulation. However, a reduction in cerebral blood flow and a certain degree of ischemia occur that are not completely compensated by the basilar arteries.

Right or left carotid ligature and sham-operation were also performed in rats with intact corpus callosum. Rats (20 animals per group) were killed 48 hr after carotid ligature and the brains were rapidly removed for microvessel purification. Tissue sections were examined for verification of corpus callosum disruption. In 90% of the animals the entire length of the corpus callosum appeared severed on the midline. Only successfully operated rats without signs of infection or hemorrhage were used for the experiments.

**Lesion of the central noradrenergic system.** A 250 µg dose of 6-OHDA (freshly dissolved in 20 µl 0.9% sodium chloride containing 1 mg/ml ascorbic acid) was injected intraventricularly under ether anesthesia. A midline incision was made through the scalp, the underlying membranes were scraped away from the skull, and a hole was made with a microdrill. The drug was injected with a microsyringe according to the following coordinates: 2 mm lateral, 2 mm posterior to bregma; the depth from the dura was 3.5 mm. Pretreatment with 25 mg/kg benztropin, which selectively blocks the uptake of 6-OHDA into dopamine neurons was performed to protect the dopaminergic system. Benztropin was administered intraperitoneally 30 min before injection of 6-OHDA.

Control rats were treated with 20 µl iv saline. After 15 days, 6-OHDA-treated and control rats underwent right carotid ligation, as described above. Animals (20 rats per group) were killed 48 hr after ligature and the brains were rapidly removed for microvessel isolation. To verify the success of the lesion, frontal cortex and striata were dissected from each brain and catecholamines were extracted according to the method of Felice et al. and assayed by high-pressure liquid chromatography.

**Isolation of cerebral capillaries and 125I binding.** Cerebral microvessels from the left and right hemispheres were isolated by an albumin flotation and glass bead isolation technique described by Kobayashi et al. The purity of the preparations was routinely controlled by phase-contrast microscopic observation and by measurement of a marker enzyme for brain capillaries, γ-glutamyltranspeptidase. The preparations appeared free from neuronal and glial elements and were mainly composed of capillaries. β-Adrenergic receptors were measured by the specific radioligand 125I-iodocyanopindolol (ICYP), as previously described.

**Results**

The maximum number of binding sites (Bmax) and the dissociation constant of the binding (Kd) were extrapolated according to Scatchard analysis. Figure 1, a, shows the effect of the right carotid occlusion in rats with intact corpus callosum on ICYP kinetic parameters. A 25% decrease in Bmax in the ipsilateral hemisphere compared with the right hemisphere of sham-operated controls and a 38% decrease of Bmax value in the contralateral hemisphere compared with the left of sham-operated controls were observed. Kd values were unmodified.

Figure 1, b, shows the effect of the right carotid occlusion in callosal-sectioned rats on ICYP binding to cerebral microvessels. A 24% decrease of Bmax values was observed in the ipsilateral hemisphere compared with the right hemisphere of sham-operated controls.
pared with the right hemisphere of sham-operated controls, whereas the decrease of Bmax in the contralateral cortex compared with the left hemisphere of sham-operated controls was 18%.

Figure 2, a, shows the effect of left carotid occlusion in rats with intact corpus callosum on ICYP kinetic variables. A 32% decrease in Bmax in the ipsilateral hemisphere compared with the left hemisphere of sham-operated controls and a 21% decrease of Bmax value in the contralateral hemisphere compared with the right of sham-operated controls were observed. Kd values were unmodified.

Figure 2, b, shows the effect of left carotid occlusion in callosal-sectioned rats. A small but nonsignificant decrease (11%) was found in the left hemisphere of ligated rats in comparison with callosal-sectioned, sham-operated controls, whereas the effect on the right hemisphere was completely abolished.

Bmax values for each experimental group are reported in table 1.

Figure 3 shows the effect of right carotid occlusion of ICYP binding to cerebral capillaries of 6-OHDA-treated rats.

The level of norepinephrine was reduced by about 63% in the frontal cortex of 6-OHDA-treated rats compared with controls (0.230 ± 0.014 and 0.089 ± 0.006 ng/mg tissue for control and treated rats, respectively), whereas the content of striatal dopamine was reduced only by 24% (10.9 ± 1 and 8.38 ± 0.6 ng/mg tissue for control and treated rats, respectively), indic-

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Right hemisphere</th>
<th>Left hemisphere</th>
<th>Right/left ratio (for Bmax)</th>
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<tbody>
<tr>
<td></td>
<td>Bmax (fmol/mg protein)</td>
<td>Kd (pM)</td>
<td>Bmax</td>
</tr>
<tr>
<td>Control</td>
<td>122 ± 11</td>
<td>69 ± 7</td>
<td>118 ± 10</td>
</tr>
<tr>
<td>Right carotid occlusion</td>
<td>91 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65 ± 5</td>
<td>73 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Corpus callosum section</td>
<td>116 ± 10</td>
<td>64 ± 6</td>
<td>112 ± 9</td>
</tr>
<tr>
<td>Corpus callosum section + right carotid occlusion</td>
<td>88 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63 ± 5</td>
<td>92 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>121 ± 10</td>
<td>62 ± 6</td>
<td>115 ± 10</td>
</tr>
<tr>
<td>Left carotid occlusion</td>
<td>96 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65 ± 5</td>
<td>78 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corpus callosum section</td>
<td>114 ± 10</td>
<td>64 ± 5</td>
<td>116 ± 10</td>
</tr>
<tr>
<td>Corpus callosum section + left carotid occlusion</td>
<td>112 ± 10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61 ± 5</td>
<td>103 ± 8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data were examined by analysis of variance followed by Tuckey’s HSD test for multiple group comparisons.

<sup>a</sup>p < .05 vs the respective nonligated control.

<sup>b</sup>p < .05 vs right hemisphere, carotid ligated.

<sup>c</sup>Not significant vs right hemisphere, callosal-sectioned, carotid ligated.

<sup>d</sup>Not significant vs the respective nonligated control.
cating that the lesion is more selective for the noradrenergic system.

The results indicate that in 6-OHDA–treated rats the effect of carotid occlusion on ICYP binding to cerebral microvessels is practically abolished in both hemispheres. In fact, a slight but nonsignificant (10%) reduction in the number of β-receptors was observed in microvessels of both left and right cortex (Bmax 134 ± 12 and 131 ± 11 fmol/mg protein for right and left hemispheres of 6-OHDA–treated rats, respectively; 117 ± 9 and 119 ± 9 fmol/mg protein for right and left hemispheres of 6-OHDA–treated, right carotid–ligated rats, respectively).

Discussion

It has been recently demonstrated that unilateral cerebral ischemia induced in gerbils and rats by occlusion of a common carotid artery leads to a reduction in the number of β-receptors in brain microvessels of both hemispheres. The observed changes in capillary receptor function may reflect concomitant events of altered neuronal and humoral regulation of brain microvasculature. The finding that the decrease in β-adrenergic receptor density is more pronounced in the left hemisphere with occlusion of either the right or left carotid artery suggests that the microvasculature of the left side of the brain is more susceptible to the effects of ischemia; however, the basis for this specific vulnerability is unknown at present. Preliminary clinical data seem also to support the view of an asymmetric responsiveness of the two hemispheres to the ischemic insult. In fact, the susceptibility of catecholaminergic neurons and their functional recovery (reflected by the level of catecholamine metabolites in the cerebrospinal fluid in patients with cerebral infarction) seem to vary in dependence on the side of the ischemic insult. In particular, in patients with left-sided hemispheric infarction the increase in catecholamine metabolites seems to be more pronounced than in patients with right-sided infarction and correlates significantly with the clinical course of the disease.

The results show that corpus callosum section partially reverses the effects induced by carotid occlusion on the contralateral hemisphere; notably, in callosal-sectioned rats the effect of left carotid ligature is also greatly attenuated in the ipsilateral hemisphere. The data provide evidence that β-adrenergic receptors located on cerebral capillaries are, at least in part, regulated by central neuronal pathways. This hypothesis is supported by anatomic and physiologic evidence, suggesting the existence of a neuronal control of brain microvasculature. In fact, ultrastructural studies have demonstrated the presence of adrenergic fibers originating from the locus ceruleus in close contact with capillary endothelial cells. A component of the noradrenergic fibers was shown to be projected to the contralateral cortex. In addition, physiologic data indicate that the stimulation or destruction of the central adrenergic system induces changes in microvascular functions. The fact that a lesion of the central noradrenergic system nearly abolishes the effects of ischemia on ICYP binding to cerebral microvessels of both hemispheres may provide further evidence that capillary β-receptors are neuronally regulated.

These findings suggest a complex pattern of cerebral asymmetry, showing a kind of “polarity” peculiar to the left side of the brain in the neuronal regulation of cerebral microvasculature. Although the basis of this phenomenon is unknown at present, the integrity of neuronal pathways connecting the hemispheres seems to contribute to both the expression and the asymmetry of the damage at the level of brain microvessels in response to ischemia, independently on the side of carotid occlusion.

The data also suggest that clinical manifestations of cerebral ischemia are not simply based on a local neural damage but reflect alterations in distant areas, which may be explained on the basis of transneuronal mechanisms.

References


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