Aging Modifies the Asymmetry in Brain Microvascular Regulation

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Cerebral ischemia induced by unilateral carotid occlusion in rats decreases in an asymmetric manner the number of \( \beta \)-adrenergic receptors in microvessels prepared from cerebral cortexes ipsilateral and contralateral to the side of the ligature. In particular, the reduction is more pronounced in the left hemisphere in case of both right and left carotid ligature. The greater receptor decrease in the left side of the brain was shown to depend on the integrity of interhemispheric connections. We show that the changes in capillary \( \beta \)-adrenergic receptors in response to unilateral carotid occlusion are qualitatively modified during aging. In particular, the asymmetry in the response pattern observed in young rats is lost. The mechanisms underlying this phenomenon may be based on an age-related impairment in the transfer of neuronal information between the two sides of the brain. (Stroke 1988;19:38-41)

The accumulation of clinical and experimental evidence has widely confirmed the existence of anatomic, biochemical, and functional asymmetries in both human and animal brain.\(^1\)\(^2\) Recent studies of cerebral circulation have raised the hypothesis that cerebral lateralization may be exerted also at the level of neuronal mechanisms regulating microvessel function.\(^3\)\(^-\)\(^5\) A large amount of experimental data suggests the existence of a functional adrenergic innervation of brain capillaries.\(^6\)\(^-\)\(^11\) The relation between vascular function and neuronal activity seems to be mediated by adrenergic receptors located on cerebral capillaries.\(^12\)\(^-\)\(^16\) The measurement of kinetic parameters of capillary \( \beta \)-adrenergic receptors in conditions of ischemia was used as a tool to investigate the asymmetry of those mechanisms that are involved in the control of the blood–brain barrier function in the two hemispheres.

In previous works it has been observed that occlusion of the right carotid artery in rats induces a decrease in the density of capillary \( \beta \)-adrenergic receptors that is more pronounced and persistent in the contralateral hemisphere.\(^5\) On the other hand, occlusion of the left carotid artery induces a greater decrease in \( \beta \)-receptor number in the ipsilateral hemisphere, suggesting that the left side of the brain is more sensitive to ischemic insult, independent of the side of carotid occlusion. The fact that resection of neuronal pathways connecting the two sides of the brain partially abolishes the effect of carotid occlusion on the contralateral hemisphere suggests that alterations induced by ischemia in distant areas may be mediated, at least in part, by transneuronal mechanisms.\(^4\)\(^5\) Recent literature suggests that aging may alter the functional differences between the two hemispheres.\(^17\)\(^-\)\(^19\) In this light, it is of interest to investigate the effect of ischemia induced by right or left carotid artery occlusion on capillary \( \beta \)-adrenergic receptors in senescent rats.

Materials and Methods

Eighteen male adult (3 months) or aged (24 months) Sprague-Dawley rats were used for each group. Under diethyl ether anesthesia, the right or left carotid artery was ligated after dissection from the accompanying nerve and vein. Sham-operated control rats underwent the same manipulation except for ligature to avoid interference in interpretation of the results. Occluded and sham-operated rats were killed 48 hours after surgery. Before and after surgery, rats were maintained in an animal care unit under a 12-hour light–dark cycle with free access to water and food. Occlusion of a common carotid artery in rats does not produce clinical signs of cerebral infarction since blood supply to the brain is also provided by basilar circulation. However, as described by Deley et al.,\(^20\) a relevant reduction of cerebral blood flow and a certain degree of ischemia occur, which are not completely compensated for by basilar vessels. Cerebral microvessels from the right and left hemispheres of the rats were isolated by the albumin flotation and glass bead filtration technique according to Kobayashi et al.\(^21\) In brief, cortices were cleaned of pial membrane and white matter. The homogenate, in oxygenated Ringer’s solution (137 mM NaCl, 5 mM KCl, 3 mM CaCl\(_2\), 12 mM NaHCO\(_3\), 1.2 mM MgCl\(_2\), 5 mM (+)-glucose, 15 mM HEPES, and 1% bovine serum albumin), was passed through nylon meshes (700 and 200 \( \mu \)m) and centrifuged at 1,000g for 10 minutes. The precipitate was suspended in Ringer’s solution containing 30% albumin and centrifuged again at 1,000g for 15 minutes. The floating layer and supernatant were resuspended and centrifuged a third time. The resulting pellets were suspended in Ringer’s solution and applied to a glass bead column. After extensive washing, microvessels attached to glass beads were released into the Ringer’s solution by agita-
tion. Purity of the preparations was routinely assessed by phase-contrast microscopy and by measurement of γ-GTP, the marker enzyme for brain capillaries. The preparations appeared to be free of neuronal and glial elements and to be composed mainly of capillaries (diameter 5–15 µm). The microvessels were collected and washed twice in 154 mM NaCl, 20 mM Tris-HCl buffer (pH 7.5) by centrifugation and were suspended in the same buffer for binding studies carried out using the specific radioligand [125I]iodocyanopindolol (ICYP) (with minor modifications of the method of Kobayashi et al12).

Recovery of microvessels per gram of cortex was the same in the occluded and sham-operated rats. However, recovery was lower in the aged than in the adult rats, possibly due to a reduction in the total number of capillaries per unit of tissue. 21

Results
The maximum number of binding sites (B_{max}) and the dissociation constant (K_d) of binding were extrapolated according to Scatchard analysis. Figure 1a shows the effect of right carotid artery occlusion in adult rats

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\text{FIGURE 1. Scatchard analysis of [125I]iodocyanopindolol specific binding to cerebral microvessels in adult (top) and aged (bottom) rats after right carotid artery occlusion. Data points are the mean of triplicate determinations from a representative experiment and varied <10%.
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\]

on ICYP kinetic parameters. A 21% decrease in B_{max} in the ipsilateral compared with the right hemisphere of sham-operated adult controls and a 34% decrease in B_{max} in the contralateral compared with the left hemisphere of sham-operated adult controls were observed. K_d was unmodified.

Figure 1b shows the effect of right carotid artery occlusion in aged rats on ICYP binding to cerebral microvessels. A 27% decrease in B_{max} was observed in the ipsilateral compared with the right hemisphere of sham-operated aged rats, whereas the difference in B_{max} in the contralateral cortex compared with the left hemisphere of sham-operated aged rats was not significant. K_d was unmodified.

Figure 2a shows the effect of left carotid artery ligation on ICYP specific binding to cerebral microvessels of adult rats. A 21% decrease in B_{max} was observed in the contralateral compared with the right hemisphere of sham-operated adult controls, whereas a 32% de-
crease was observed in the ipsilateral compared with the left hemisphere of sham-operated adult controls. No difference was found in \( K_d \). Figure 2b shows the effect of left carotid artery occlusion in aged rats on ICYP kinetic parameters. No significant decrease was observed in either right or left hemispheres of occluded aged rats compared with sham-operated aged controls.

\( B_{max} \) for each experimental group is reported in Table 1.

**Discussion**

Aging is associated with modifications of both neuronal and vascular activity. In fact, a number of studies report changes in neurotransmitter function in various cerebral areas during senescence. Catecholaminergic transmission seems to be particularly affected by advanced age, as suggested by several observations on cerebral catecholamine receptors and metabolism.\(^{23-24}\) Both human and animal studies indicate that there is no evidence for a relevant reduction in cerebral blood flow and metabolism in healthy senescence, suggesting that the equilibrium between neuronal and vascular function still operates in advanced age.\(^{25,26}\) However, it is possible that the regulatory mechanisms of microcirculation may become vulnerable to particular demands of energy and metabolic supply to the brain.\(^{27}\) Along this line, the biochemical changes in brain microvessels observed during aging, such as the decreased number of capillary \( \beta \)-receptors associated with a diminished response of adrenyle cyclase to norepinephrine,\(^{28}\) may be involved in the altered response to peculiar metabolic requirements.

The concomitant events of altered patterns of neuronal function and vascular regulation may be the basis of the qualitative changes in brain microvessel function (measured in terms of \( \beta \)-adrenergic receptor kinetic parameters) to ischemia during aging. In fact, results show a lack of effect of right carotid artery occlusion on a number of \( \beta \)-adrenergic receptors in the left hemisphere of aged rats, whereas the decrease in receptor density in the ipsilateral hemisphere is similar or even more pronounced compared with adult rats. On the other hand, no effect of left carotid artery ligature in aged rats was observed on ICYP binding to capillaries of both hemispheres. Although the basis of this phenomenon is at present unknown, for the interpretation of the data it is important to emphasize that these results are reminiscent of those previously obtained in split-brain rats.\(^{29}\) Along this line, the lack of effect of right carotid artery occlusion on the left hemisphere in aged rats suggests an impairment of the transfer of neuronal information to the contralateral side of the brain. Furthermore, the fact that the effect of left carotid artery ligature was also greatly attenuated in the ipsilateral hemisphere leads to the hypothesis that mechanisms regulating microvessel response to ischemia, which in adults seems to be exerted in an asymmetric manner showing a kind of polarity of the left side of the brain, depend on preserved neuronal integrity. However, the possibility that other factors, such as circulating catecholamines or changes in perfusion pressure, may contribute to the observed modifications in \( \beta \)-receptors cannot be ruled out.

These data are in line with recent evidence in the literature suggesting that neurochemical asymmetries existing at birth may change with age.\(^{18,19}\) According to these authors, aging seems to alter the asymmetries of the hemispheric functions as far as neurophysiologic capacities or the control of sensory and pain thresholds.

Our data may contribute to the development of new perspectives for investigating the aging brain, suggesting that senescence may be associated with concomitant changes in vascular function and neuronal pathways and could be of potential interest for the study of clinical aspects of cerebrovascular disease in the elderly.

**Table 1.** \( B_{max} \) of \([^{125}I] \)iodocyanopindolol Binding to Cerebral Microvessels After Right or Left Carotid Artery Occlusion in Adult and Aged Rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Sham operation</td>
<td>118 ± 11</td>
<td>116 ± 11</td>
</tr>
<tr>
<td>Adult</td>
<td>Right carotid artery occlusion</td>
<td>93 ± 8*</td>
<td>77 ± 7†</td>
</tr>
<tr>
<td>Aged</td>
<td>Sham operation</td>
<td>92 ± 9†</td>
<td>90 ± 8‡</td>
</tr>
<tr>
<td>Aged</td>
<td>Right carotid artery occlusion</td>
<td>67 ± 7*</td>
<td>93 ± 9§</td>
</tr>
<tr>
<td>Adult</td>
<td>Sham operation</td>
<td>120 ± 11</td>
<td>117 ± 11</td>
</tr>
<tr>
<td>Adult</td>
<td>Left carotid artery occlusion</td>
<td>95 ± 8*</td>
<td>80 ± 8‡</td>
</tr>
<tr>
<td>Aged</td>
<td>Sham operation</td>
<td>94 ± 8‡</td>
<td>93 ± 8§</td>
</tr>
<tr>
<td>Aged</td>
<td>Left carotid artery occlusion</td>
<td>88 ± 8§</td>
<td>86 ± 7§</td>
</tr>
</tbody>
</table>

Data are mean ± SD (fmol/mg protein) of 4 experiments and were compared using analysis of variance followed by Tukey's honestly significant difference test for multiple group comparison. \( K_d \) values, ranging from 58 to 68 pM, were unmodified.

* \( p < 0.05 \) vs. respective sham operation.
† \( p < 0.05 \) vs. right hemisphere, carotid occlusion.
‡ \( p < 0.05 \) vs. respective adult, sham operation.
§ Not significant vs. respective aged, sham operation.

**References**


**KEY WORDS** • aging • rats • microcirculation • cerebral ischemia
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