Time-Related Asymmetric Changes of Brain Microvessel β-Adrenergic Receptors in the Two Hemispheres After Carotid Occlusion

MARIA SANDRA MAGNONI, PH.D., LUDOVICO FRATTOLA, M.D.,* GIULIO PASINETTI, PH.D., STEFANO GOVONI, PH.D., FIORENZO BATTAINI, PH.D., and MARCO TRABUCCHI, M.D.†

SUMMARY The effect of short term and long term ischemia induced by right carotid occlusion was studied on β-adrenergic receptor function in rat cerebral microvessels. The results show a different time-dependent responsiveness of the two hemispheres to ischemia, with a pronounced and more persistent decrease in the number of capillary β-receptors in the left side of the brain. The data suggest the existence of asymmetries in the control of brain microvasculature which may mediate the different time-course of β-receptor changes in response to ischemia.

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Recent studies demonstrated a different responsiveness of the microvasculature of the two hemispheres to ischemia. In fact, 48 h occlusion of the right carotid artery in the rat and in the gerbil induces a reduction in the number of β-receptors in brain capillaries of both hemispheres; notably, the reduction in the contralateral hemisphere is more marked than in the ipsilateral one, suggesting a different sensitivity of the microvasculature of the two sides of the brain to ischemia. To further investigate the responsiveness of the microvasculature of the two hemispheres to ischemia and the changes of its regulatory mechanisms, the natural evolution of the phenomenon was studied by measuring the effect of right carotid ligation on β-adrenergic receptors in brain capillaries at early (2 to 38 h) and late times (96 h and 14 days) after the occlusion.
Methods

Adult male Sprague Dawley rats (200 g) were used. The animals (15 per group) were anesthetized with chloral-hydrate (350 mg/kg) and the right carotid artery was exposed through a ventral midline cervical incision. Two surgery threads were placed around the artery after dissection from the vagus nerve and vein to form two loosened ligatures. The incision was closed leaving the ends of the threads accessible externally. Rats were treated with antibiotic and caged individually for 24 h. The carotid artery of each animal was occluded by pulling and clamping the ends of the threads. Sham-operated rats underwent the same kind of manipulation at the carotid level except for ligature. Rats were sacrificed after 2, 6, 12, 24, 48, 96 h and 14 days of carotid occlusion or sham-operation. The success of the ligature was verified in each animal after sacrifice. In the rat, the occlusion of a common carotid artery does not produce clinical signs of cerebral infarction, since blood supply to the brain is also provided by the basilar circulation. However, a reduction in blood flow and a certain degree of ischemia occur20 which are not completely compensated by basilar vessels. Cerebral microvessels from the left and right hemispheres of carotid-ligated and sham-operated controls were isolated by albumin flotation and glass bead filtration technique according to Kobayashi et al.13 The purity of the preparations was confirmed by phase-contrast microscopy observation.13 β-adrenergic receptors were measured using the specific radioligand 125I-iodocyanopindolol (ICYP).13 The recovery of microvascular proteins per group of cortical tissue was the same in each experimental group.

Results

The maximum number of binding sites (Bmax) and the dissociation constant of the binding (Kd) were extrapolated according to Scatchard analysis.21 Bmax and Kd values are the mean ± S.D. of 4 experiments carried out in triplicate, which varied less than 10%. Student t-test was used to analyze the data.

Figure 1a shows the effect of 2 h right carotid occlusion on ICYP binding to brain microvessels. A 29% (p < 0.01) decrease of Bmax in the ipsilateral hemisphere compared with the right hemisphere of sham-operated controls is observed, whereas no change is detected in the contralateral hemisphere (Bmax for right hemispheres: 117 ± 10 and 83 ± 8 fmol/mg prot. for control and ligated rats, respectively; Bmax for left hemispheres: 114 ± 9 and 111 ± 10 fmol/mg prot. for control and ligated rats, respectively).

Figure 1b shows the effect of 12 h occlusion of the right carotid artery on ICYP kinetic parameters. A 35% (p < 0.01) decrease in Bmax in the ipsilateral hemisphere compared with the right hemisphere of sham-operated controls and a slight decrease (10%) of Bmax in the contralateral hemisphere in comparison with the left one of sham-operated controls were observed (Bmax for right hemispheres: 120 ± 11 and 78 ± 8 fmol/mg prot. for control and ligated rats, respectively; Bmax for left hemispheres: 118 ± 10 and 106 ± 10 fmol/mg prot. for control and ligated rats, respectively).

Figure 1c shows the effect of 48 h occlusion of the right carotid artery on ICYP binding to brain microvessels. Bmax value in the ipsilateral hemisphere is decreased by 21% (p < 0.05), whereas a more pronounced decrease of Bmax (32%) (p < 0.01) is observed in the contralateral hemisphere (Bmax for right hemispheres: 123 ± 11 and 97 ± 9 fmol/mg prot. for control and ligated rats, respectively; Bmax for left hemispheres: 118 ± 11 and 80 ± 8 fmol/mg prot. for control and ligated rats, respectively).

Figure 1d shows the effect of 14 days occlusion of 

![Figure 1. Scatchard analysis of ICYP specific binding to rat cerebral microvessels after 2 (a), 12 (b), 48 (c) hours and 14 days (d) of carotid occlusion. Points shown in the figures are from representative experiments and are the mean of triplicate determinations which varied less than 10%.](http://stroke.ahajournals.org/Downloaded from http://stroke.ahajournals.org/ by guest on September 10, 2017)
the right carotid artery on ICYP binding to brain microvessels. Bmax value in the ipsilateral hemisphere is now comparable to that of the right hemisphere of sham-operated controls, whereas a 27% ($p < 0.01$) decrease in Bmax value is still observed in the left hemisphere in comparison with the left one of sham-operated controls (Bmax for right hemispheres: 124 ± 12 and 123 ± 11 fmol/mg prot. for control and ligated rats, respectively; Bmax for left hemispheres: 119 ± 11 and 86 ± 8 fmol/mg prot. for control and ligated rats, respectively).

Kd values were unchanged in each experiment, ranging from 60 to 70 pM.

Figure 2 shows the complete time course of the phenomenon, expressed as % of decrease in ICYP binding to brain microvessels of carotid-ligated rats in comparison with the respective controls, for right and left hemispheres separately. Additional experiments on the effect of ischemia at 6, 24, 96 h were performed. Bmax values for right hemispheres were 76 ± 8, 82 ± 8 and 123 ± 11 fmol/mg prot. for 6, 24, 96 h right carotid occlusion, respectively. Bmax values for left hemispheres were 109 ± 10, 100 ± 9, 84 ± 8 fmol/mg prot. for 6, 24, 96 h right carotid occlusion, respectively. Kd values were unmodified and ranged from 60 to 70 pM.

**Discussion**

The results show a time-related difference in the responsiveness of the two hemispheres to ischemia induced by right carotid occlusion. At early time ischemia causes a marked decrease in the number of β-adrenergic receptors in brain capillaries of the ipsilateral hemisphere, which returns to control values within few days. In the contralateral hemisphere, no change is observed in the first hours after carotid occlusion, whereas a pronounced and long lasting reduction may be observed after 48 h ischemia, still persisting after 14 days.

Various experimental observations suggest the existence of a neuronal control of brain microvessels. In fact, ultrastructural data have demonstrated the presence of adrenergic fibers originating from the locus coeruleus in close contact with capillary endothelial cells, while physiological studies indicate that the stimulation or destruction of the central adrenergic system induce changes in cerebral microvessel function. Furthermore, the section of corpus callosum was shown to partially reverse capillary receptor decrease induced by right carotid occlusion in the left hemisphere.

These data provide evidence that β-adrenergic receptors located on brain microvessels are regulated also by neuronal pathways. Thus, changes induced by ischemia in the function of catecholaminergic neurons supplying microvessels may account for the observed reduction of β-adrenergic receptor number after carotid occlusion. In this light, the alterations induced by ischemia in near and remote areas of the brain may be explained, at least in part, on the basis of transneuronal mechanisms. Along this line, the present data showing a different time-dependent susceptibility of the two hemispheres to ischemia, with a pronounced and more persistent decrease in β-receptor density in the left side, support the hypothesis for the existence of an asymmetric control of brain microvasculature, which may mediate the different time course of the capillary receptor changes after the ischemic insult. On the other hand, there is the possibility that the reduction of β-adrenergic receptors in capillaries may be ascribed not only to neuronal mechanisms but also to humoral factors, such as alterations of cerebral blood flow and metabolisms leading to changes in blood catecholamine levels, which occur during ischemia even in the hemisphere contralateral to the injury.

Preliminary clinical data are also suggestive of an asymmetric neurochemical pattern of the two hemispheres in response to ischemia. In fact, the vulnerability of catecholaminergic neurons to hypoxia and their functional recovery (reflected by the content of catecholamine metabolites in the cerebrospinal fluid of patients with cerebral infarction) seem to vary in dependence on the side of the ischemic injury. Although the clinical relevance of the phenomenon is at present unknown, the data suggest that neurological manifestations of cerebral ischemia may be not only based on a local neuronal damage, but reflect more complex events involving distant brain areas.

**References**

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