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**Effect of Common Carotid Occlusion on β-Adrenergic Receptor Function in Cerebral Microvessels**

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**SUMMARY** β-adrenergic receptors were measured in cerebral microvessels of gerbils and rats after ligation of the right or left common carotid artery. The results indicate a decrease in the number of β-adrenergic receptors in brain microvessels of both ipsilateral and contralateral hemispheres. This event may reflect altered patterns of the neuronal regulation of brain microvasculature and may be related to cerebrovascular alterations which are concomitant with ischemia. Furthermore, the results show that the decrease in β-receptor density is more pronounced in the left hemisphere, independently on the side of carotid occlusion. This finding suggests that microvessel function in the left side of the brain is more vulnerable to hypoxia effects.

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**CEREBRAL ISCHEMIA** due to vasospasm, thrombi or emboli results in a series of events leading to neuronal necrosis and failure of synaptic transmission. A number of studies point to a role for catecholamines in the pathophysiology of brain injury following cerebral ischemia. In fact, massive amounts of catecholamines are released by ischemic neurons, as suggested by histochemical studies and by the increased level of biogenic amines in the cerebrospinal fluid of humans affected by cerebral infarction.\(^1\)\(^2\) This event may contribute to the development of ischemic brain damage. Alterations in catecholamine content and metabolism associated with changes in receptor number, affinity and coupling to adenylate cyclase have been reported in various cerebral areas after experimental vascular lesions.\(^6\)\(^-\)\(^13\) These biochemical and histologi-
tical changes are accompanied by alterations of brain microvascular function. In fact, increased capillary permeability to water, leading to cerebral edema formation, and decreased glucose uptake and O₂ consumption have been reported in experimental ischemia.¹¹,¹²–¹⁷

Increasing evidence suggests the existence of neuronal controls of cerebral microvasculature, mostly exerted by central adrenergic neurons. In fact, ultrastructural studies have revealed the existence of adrenergic fibers originating from the locus coeruleus and innervating small brain vessels, whereas physiological experiments indicate that the destruction or stimulation of the central noradrenergic system may alter blood-brain barrier permeability and cerebral blood flow.¹⁸–²²

This hypothesis is further supported by the identification in cerebral microvessels of β-adrenergic receptors coupled to adenylate cyclase, which participate to the regulation of capillary function.²³–²⁹

In order to investigate the involvement of β-adrenergic receptors in brain microvascular alterations which occur in cerebral ischemia, we have measured β-adrenergic receptors in preparations of cerebral microvessels obtained from gerbils and rats after ligation of right or left common carotid artery.

Methods

Mature male and female Mongolian gerbils (60–70 g) and adult male Sprague-Dawley rats (200 g) were used for the experiments. Mongolian gerbils provide a good experimental model for the study of cerebral infarction. Unlike other rodents, gerbils lack the circle of Willis. Thus, ligation of a common carotid artery causes unilateral cerebral infarction in a large percentage of animals. A 50% mortality rate is observed within 5 days of ligation.³⁰

Mongolian gerbils were anesthetized lightly with diethyl ether and the right or left common carotid was exposed through a ventral midline cervical incision. After dissection from its accompanying vagus nerve and vein, the carotid was rapidly double ligated with a 4-0 silk suture and cut between ligatures. About 50–60% of the animals exhibited clinical signs of infarction, including hypokinesia of the contralateral side of the body and ipsilateral circling behavior. In sham-operated animals, the carotid was exposed but not ligated. Sham-operated and symptomatic animals were sacrificed 24 h after lesion (about 20 animals per group). Cerebral microvessels were isolated from gerbils and rats after ligation of right or left common carotid artery.

The purity of the preparations was confirmed by phase-contrast microscopy observation and by measurement of γ-glutamyltranspeptidase (γ-GTP), a marker enzyme of brain capillaries.³² The preparations were predominantly composed of capillaries, and free from neuronal and glial elements.³³ β-adrenergic receptors were measured using the specific radioligand lodohydroxybenzylpindolol (IHYP).³⁴

Protein content was measured according to Lowry et al.³⁵ The recovery of microvascular proteins per g of cortex is the same in all the preparations (data not shown).

Results

IHYP binding to cerebral microvessels was performed as described by Kobayashi et al.³³ The maximum number of binding sites (Bmax) and the dissociation constant (Kd) of the binding were extrapolated according to Scatchard analysis.³² Student t-test was used to analyze the data.

Figure 1a shows the effect of the occlusion of the right carotid artery in gerbils on IHYP binding to cerebral microvessels. A 32% reduction (p < 0.01) of Bmax value in the ipsilateral hemisphere of lesioned gerbils versus the right hemisphere of sham-operated controls and a 49% reduction (p < 0.001) of Bmax value in the contralateral hemisphere of lesioned animals versus the left hemisphere of sham-operated controls were observed (130 ± 11 and 126 ± 10 fmol/mg protein for right and left hemisphere of sham-operated gerbils, respectively; 88 ± 9 and 65 ± 5 fmol/mg protein for right and left hemisphere of lesioned gerbils respectively), while Kd values are comparable (64 ± 3 and 66 ± 4 pM for right and left hemisphere of sham-operated gerbils, respectively; 60 ± 5 and 56 ± 4 pM for right and left hemisphere of lesioned gerbils, respectively).

The ligature of the left carotid (fig. 1b) induced a 48% reduction (p < 0.001) of Bmax value in the ipsilateral hemisphere of lesioned gerbils versus the left hemisphere of sham-operated controls and a 30% reduction (p < 0.01) of Bmax value in the contralateral hemisphere of lesioned animals versus the right hemisphere of sham-operated controls (131 ± 11 and 124 ± 12 fmol/mg protein for right and left hemisphere of sham-operated gerbils, respectively; 91 ± 9 and 64 ± 6 fmol/mg protein for right and left hemisphere of lesioned gerbils, respectively), without any changes in Kd values (61 ± 4 and 64 ± 3 pM for right and left hemisphere of sham-operated gerbils, respectively; 60 ± 5 and 55 ± 4 pM for right and left hemisphere of lesioned gerbils, respectively).

Comparable results were obtained with rats. Figure 2a shows the effect of the ligature of the right carotid on IHYP binding to rat cerebral microvessels. Bmax value in lesioned rats was decreased of 25% (p < 0.01) in the ipsilateral hemisphere compared to the right hemisphere of sham-operated controls, and of 35% (p
FIGURE 1. Scatchard analysis of IHYP specific binding to cerebral microvessels of gerbils after ligature of the right (a) and left (b) common carotid artery.

- right hemisphere of lesioned gerbils; □ left hemisphere of lesioned gerbils; • • right hemisphere of sham-operated controls; O O left hemisphere of sham-operated controls.

Values are from a representative experiment and are the mean of triplicate determinations which varied less than 10%.

< 0.01) in the contralateral hemisphere compared to the left hemisphere of sham-operated controls (117 ± 11 and 112 ± 11 fmol/mg protein for right and left hemisphere of sham-operated rats, respectively; 87 ± 8 and 73 ± 6 fmol/mg protein for the right and left hemisphere of lesioned rats, respectively), while Kd values were unmodified (69 ± 4 and 65 ± 5 pM for right and left hemisphere of sham-operated rats, respectively; 67 ± 6 and 70 ± 6 pM for right and left hemisphere of lesioned rats, respectively).

Figure 2b indicates that the ligature of the left carotid induces a 32% reduction (p < 0.01) of Bmax value in the ipsilateral hemisphere compared to the left hemisphere of control rats and 22% reduction (p < 0.02) in the contralateral hemisphere compared to the right hemisphere of controls (114 ± 10 and 118 ± 9 fmol/mg protein for right and left hemisphere of sham-operated rats, respectively; 89 ± 8 and 80 ± 7 fmol/mg protein for right and left hemisphere of lesioned rats, respectively), without any changes of Kd values (60 ± 5 and 62 ± 6 pM for right and left hemisphere of sham-operated rats, respectively; 65 ± 5 and 69 ± 5 pM for right and left hemisphere of lesioned rats).

Discussion

The results indicate that ligature of one common carotid artery in gerbils and in rats is followed by β-receptor number decrease in cerebral microvessels of both ipsilateral and contralateral hemispheres. The extent of such reduction is more marked in gerbils than in rats. Although the mechanisms leading to the decrease in β-receptor density are presently unknown, this event may reflect an altered pattern of adrenergic neuron activity controlling brain microvasculature which occurs during prolonged ischemia.

The loss of binding sites may be due to massive release of catecholamines from ischemic neurons into the synaptic cleft. This hypothesis does not explain the major impairment of β-receptor function in cerebral
microvessels of the contralateral cortex. In particular, the results indicate that in case of ligature of the right carotid artery the decrease of β-receptor number is more pronounced in the contralateral hemisphere, both in gerbils and rats. Previous studies have reported the peculiar phenomenon, known as diaschisis, in which the local cerebral damage due to ischemia causes functional depression and biochemical changes in areas of the brain distant from the site of injury. 4, 6, 10, 11, 35 Although the basis of this phenomenon has never been fully understood, there is the possibility that nerve pathways connecting the cerebral hemispheres play a role in the biochemical and functional alterations observed in distal brain regions. In particular, it has been demonstrated that the locus coeruleus, the cellular origin for cortical noradrenergic fibers which innervate intraparenchymal vessels, projects to wide areas of the cortex including a component to the contralateral side. 35, 37 In this view, the impairment of β-receptor function in cerebral microvessels of the contralateral hemisphere may be, at least in part, explained on the basis of a transneuronal mechanism. The hypothesis is further supported by preliminary results indicating that the reduction of β-receptor number in the left cortex after right carotid occlusion is partially reversed by corpus callosum section, while the effect on the ipsilateral hemisphere is maintained. 38

The impairment of adrenergic regulatory mechanisms of brain microvasculature in both injured and uninjured cerebral cortex indicate that clinical manifestations of ischemia cannot be explained solely on the basis of local neuropathology but may reflect alterations occurring in the whole brain. It is important to note that the reduction of β-receptors is more marked in the left hemisphere, both in case of ligature of the left and right carotid artery. This fact is more marked in the left hemisphere, both in case of ligature of the right carotid artery. This fact is more marked in the left hemisphere, both in case of ligature of the right carotid artery. This fact is more marked in the left hemisphere, both in case of ligature of the right carotid artery.

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