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Research Report

Astrocytes react to oligemia in the forebrain induced by chronic bilateral common carotid artery occlusion in rats

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Abstract

The effects of oligemia (moderate ischemia) on the brain need to be explored because of the potential role of subtle microvascular changes in vascular cognitive impairment and dementia. Chronic bilateral common carotid artery occlusion (BCCAO) in adult rats has been used to study effects of oligemia (hypoperfusion) using neuropathological and neurochemical analysis as well as behavioral tests. In this study, BCCAO was induced for 1 week, or 2, 4, and 6 months. Sensitive immunohistochemistry with marker proteins was used to study reactions of astrocytes (GFAP, nestin), and lectin binding to study microglial cells during BCCAO. Overt neuronal loss was visualized with NeuN antibodies. Astrocytes reacted to changes in the optic tract at all time points, and strong glial reactions also occurred in the target areas of retinal fibers, indicating damage to the retina and optic nerve. Astrocytes indicated a change in the corpus callosum from early to late time points. Diffuse increases in GFAP labeling occurred in parts of the neocortex after 1 week of BCCAO, in the absence of focal changes of neuronal marker proteins. No significant differences emerged in the cortex at longer time points. Nestin labeling was elevated in the optic tract. Reactions of microglia cells were seen in the cortex after 1 week. Measurements of the basilar artery indicated a considerable hypertrophy, indicative of macrovascular compensation in the chronic occlusion model. These results indicate that chronic BCCAO and, by inference, oligemia have a transient effect on the neocortex and a long-lasting effect on white matter structures.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Oligemia; Astrocytes; Vascular dementia; Retina

1. Introduction

Most experimental studies on cerebral vascular disease have focussed on models of global or focal ischemia that typically induce severe reductions of cerebral blood flow (CBF) and serious neuronal loss or tissue infarction [19,53,54]. Much less is known about the effects of a moderate reduction of CBF which is defined as oligemia (also termed “moderate ischemia”). A moderate reduction of CBF in the range of 40–60% of control levels is usually not associated with tissue damage [19,27,43], although there may be subtle effects on neurons and glial cells. These considerations for oligemia relate to subtle changes due to vascular disease in the aging brain, i.e., in vascular cognitive impairment, vascular dementia, or “mixed” dementia [6,15,21,24]. Cerebral hypoperfusion has been also invoked as a mechanism in APP overproduction [4,23,66] and in Alzheimer’s disease [12,15,24], although this issue is complex and controversial [21,44].

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Bilateral common carotid artery occlusion (BCCAO) leads to a moderate reduction of CBF in the forebrain of adult rats into the oligemic range [7,11,15,20,22,33,36,37,38,51,59]. BCCAO is tolerated by adult rats due to an effective collateral flow through the basilar artery and the arterial circle of Willis. Several biochemical studies have indicated acute, subtle changes after BCCAO [11,32,60,62,64]. In a preceding study, we used the expression of brain-derived neurotrophic factor (BDNF) mRNA as an indicator for a neuronal stress response in the hippocampus after BCCAO [51]. Chronic BCCAO has been used in behavioral studies as an abstracted model of reduced cerebral perfusion ("chronic hypoperfusion") in aging [3,11,12,15,31,38,57,72]. However, the interpretation of behavioral tests involving visual functions is problematic due to concomitant damage to the retina, and possibly to the optic nerve [8,55]. In young rats, the chronic vascular occlusion leads to various compensatory changes in the vasculature [34], and CBF tended to return to normal levels after several weeks [36]. Preliminary evidence for an enlargement of the basilar artery in the BCCAO model, indicating macrovascular compensation, was obtained [50].

The aim of the present study was to test whether chronic BCCAO, and by inference oligemia, can induce changes in glial cells as an indicator of tissue reactions. Progressive histological changes were reported for the hippocampus of middle-aged rats subjected to BCCAO [3,38], whereas other studies did not find progressive tissue damage in younger rats with chronic occlusions [9,42]. Unilateral focal lesions were also reported in the BCCAO model [17]. Differences in the outcome after BCCAO in different laboratories may be explained by the rat strain and the age of animals at the time of occlusion. Most studies have shown changes in the cerebral white matter which suffered from more severe hypoperfusion during BCCAO than the neocortex [16,58,67–70]. The hippocampus was largely devoid of neuronal damage up to 2 months after BCCAO in our preceding study [51]. Additional observations made on hematoxylin–eosin (H&E)-stained sections in the forebrains of rats surviving between 1 and 12 months of BCCAO indicated that only some animals (below 20%) had focal lesions; in the majority of animals, the histological analysis revealed an overall normal architecture in the forebrain (unpublished observation). Thus, if neuronal changes are caused by oligemia in the BCCAO model, they are mostly beyond the limits of detection by qualitative light microscopy in conventionally stained sections. Astrocytes and microglial cells are sensitive indicators of tissue pathology, and the reaction of these cells may provide an index for a subtle change in oligemia.

Glial fibrillary acidic protein (GFAP) is commonly used as a sensitive marker of changes in astrocytes. Previous studies showed GFAP-immunoreactive astrocytes in areas of overt ischemic neuronal injury after global brain ischemia [41,48]. Immunohistochemical markers may also indicate an activation of glial cells in the absence of overt neuronal damage, e.g., in spreading depression and mild seizure activity [5,28]. Chronic hypoxia can be associated with glial reactions in the absence of obvious neuronal loss in rats [74]. Markers for astrocytes should be suitable to trace out subtle changes in reaction to BCCAO. Previous studies on BCCAO reported changes in GFAP labeling in the hippocampus, white matter regions, and visual pathways [9,16,38,57,67]. Most of these studies used paraffin sections which demonstrated reactive astrocytes by GFAP antibodies in areas with neuropathological changes. A differentiated pattern of GFAP labeling emerges in the normal rat brain when vibratome sections are used with a free-floating approach [46,47,49,65], and subtle changes can be detected under pathological conditions [46,65]. Nestin is only expressed in reactive astrocytes in areas with severe neuronal degeneration [14]. Activation of microglial cells is another sensitive parameter for subtle pathology, and microglial activation has been studied in a model of reversible BCCAO [1], or in conjunction with white matter damage in chronic BCCAO [16,29].

We here studied changes of astrocytes and microglial cells between 1 week and 6 months of chronic BCCAO. Changes in immunoreactivity for GFAP, nestin, and the neuronal marker NeuN were examined. Isolectin binding in microglial cells was studied in parallel. Changes of the basilar artery were measured as an indicator for macrovascular compensatory changes after BCCAO.

2. Materials and methods

2.1. Occlusion model

Bilateral common carotid artery occlusion (BCCAO) was used to induce a moderate reduction of cerebral blood flow to the forebrain, i.e., oligemia [51]. Protocols were approved by the University of Miami's Animal Care and Use Committee. Adult male Wistar rats (body weight 250–350 g) were anesthetized in 3% halothane in 70% N_2O and balance of O_2. Food was withheld overnight before surgery in order to stabilize plasma glucose. Following a midline incision, both common carotid arteries were dissected free and doubly ligated with 3-0 silk. After occlusion, all animals appeared overtly normal and did not display grossly abnormal behavior or seizures. Sham controls had anesthesia and surgical exposure. The time points studied were 1 week, and 2, 4, and 6 months after occlusion, with $n = 6–7$ animals subjected to BCCAO, and $n = 3$ to sham occlusions at each time point. After different survival periods, brains were fixed by perfusion with 4% neutral-buffered paraformaldehyde, removed from the cranium, post-fixed for about 24 h, and then stored in PBS at +4 °C.