CHANGES IN STATE OF THE GLIA IN DIFFERENT PARTS OF THE RAT BRAIN AFTER SYSTEMIC CIRCULATORY ARREST

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UDC 615-036.882-036.8-07:616.831.171-081.1

KEY WORDS: systemic circulatory arrest; astrocytes; glial fibrillary acidic protein

The most characteristic properties of glia include reactive hypertrophy and an increase in the content of glial fibrillary acidic-protein (GFAP) in the astrocytes in response to brain damage. GFAP is the main component of glial intermediate filaments, normally found in fibrous but not in protoplasmic astrocytes [11]. In various neurological diseases [10, 12] or their experimental models [9, 14, 15], and also after mechanical brain injury [6, 13], the immunoreactivity (IR) of GFAP is increased. Consequently, the presence of AGFP is a reliable marker of reactive astrocytes in the widest possible range of pathological states of the brain.

The aim of this investigation was, by using immunocytochemical detection of GFAP-expressing astrocytes, to assess changes in the state of the glia in the postresuscitation period, and to compare them with the duration of systemic circulatory arrest, the degree of recovery of the neurological status of the resuscitated animals, and the severity of morphological changes in the neurons.

EXPERIMENTAL METHOD

The investigation was conducted on 10 noninbred mature male albino rats weighing 160-180 g, subjected to systemic circulatory arrest, and three intact rats of the same sex and age (control). Systemic circulatory arrest for 10 or 15 min was induced in the rats under ether anesthesia by intrathoracic compression of the vascular bundle of the heart [3]. Resuscitation was carried out by indirect cardiac massage combined with artificial ventilation of the lungs with air. In the course of resuscitation the times of recovery of cardiac activity, spontaneous respiration, and corneal reflexes were recorded. The neurological status of the animals was assessed on a 100-point scale. Including 19 parameters (character of respiration, response to pain, disturbance of static function, etc.) [4]. A score of 100 points corresponded to brain death, whereas 0 corresponded to complete disappearance of the neurological deficit.

The animals were killed by decapitation under ether anesthesia 4 and 7 days after resuscitation and the tissue of their cerebellum, hippocampus, and sensomotor cortex was removed for investigation. After fixation in alcohol and embedding in paraffin wax sections 5-6 μ were cut and stained with cresyl violet by Nissl's method. Parallel sections were treated for immunocytochemical detection of GFAP by a modified three-step peroxidase-antiperoxidase (PAP) method [7]. Monoclonal antibodies were obtained from "ICN Immunological" (USA; clone G-A5).

EXPERIMENTAL RESULTS

All rats surviving systemic circulatory arrest for 10 min had full but delayed recovery of their neurological status (later than the 1st day after resuscitation). They still had neurological disturbances (4-6 points) 1 and 2 days after resuscitation, and these did not disappear until the 3rd day of the postresuscitation period (0 point).

Institute of General Resuscitation, Russian Academy of Medical Sciences, Moscow. (Presented by Academician A. V. Negovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 176-179, August, 1992. Original article submitted February 6, 1992.

0007-4888/92/0008-1151$12.50

Rats surviving systemic circulatory arrest for 15 min had marked neurological disturbances (8 points) 3 days after resuscitation, and these lasted for 4 days of the postresuscitation period, i.e., these animals were characterized by permanent disturbances of their neurological status.

In animals surviving systemic circulatory arrest for 10 min, moderately severe morphological changes without any visible foci of disappearance of nerve cells were observed in all parts of the brain studied. On the 4th day after resuscitation, degenerative changes in the neurons were found in cortical layers V-VI and, less frequently, II-III, in the CA4 sector, and sometimes in sectors CA2-CA3 of the hippocampus, and also in the Purkinje layer of cells in the cerebellum. By the 7th day after resuscitation of the animals there were far fewer altered neurons, and they were mainly found in layer V of the sensomotor cortex, the CA4 sector of the hippocampus, and among the Purkinje cells of the cerebellum. Thus in the course of the postresuscitation period, in animals with no neurological deficit the state of the brain improved, evidently due to the development of compensatory and reparative changes [2].

In rats surviving systemic circulatory arrest for 15 min, on the 4th day after resuscitation multiple degenerative changes were found in the neurons, with foci of absence of nerve cells in layers V and II-III of the cerebral cortex, in sectors CA3-CA4, and in the dentate gyrus of the hippocampus and the layer of Purkinje cells in the cerebellum. Consequently, an increase in the duration of circulatory arrest led to the onset of more severe and more widespread changes in neurons and the development of a process of disappearance of nerve cells, which evidently provided a basis for the formation of lasting neurological disorders in the animals.

Immunohistochemical investigation showed that there was a sharp increase in immunoreactivity (IR) of the GFAP in fibrous astrocytes of the white matter of the brain, and IR of GFAP of astrocytes appeared in the gray matter. Analysis of previous data obtained on a model of isolated cerebral ischemia [15] revealed that the severity of IR of GFAP depends essentially on the phase of the postresuscitation process. For instance, on the 4th day after circulatory arrest for 10 min, concentrations of IR of GFAP of astrocytes were observed in layers I and VI of the sensomotor cortex, among the granulecells of the dentate gyrus, and pyramidal neurons of sectors CA3-CA4 of the hippocampus, and also in the granular layer of the cerebellum (Fig. 1a, b, c). By the 7th day of the postresuscitation period IR of GFAP was reduced in the white matter of the brain, although it still exceeded the control level (Fig. 1d, e). A few IR GFAP of astrocytes were found only in cortical layer VI, hippocampal sector CA4 and, to a lesser degree, the granular layer of the cerebellum (Fig. 1f, g). Reduction of IR of GFAP in the later stage of the post-resuscitation period in animals with neurological recovery may have been connected with normalization of the state of the brain, as shown by the results of pathomorphological analysis. However, it is possible that towards the 7th day after resuscitation, inhibition of protein synthesis may take place in the astrocytes, in the same way as has been demonstrated for neurons [1].

In rats recovering from systemic circulatory arrest lasting 15 min, IR of GFAP was significantly higher in all parts of the brain studied than in the animals after circulatory arrest for 10 min (Fig. 2). Numerous hypertrophied astrocytes with high IR of GFAP were found not only in cortical layers I and VI, and the granular layer of the cerebellum, but also in all parts of the hippocampus. Consequently, the intensity of the reaction of the astrocytes in the form of an increase in IR of GFAP depended essentially on the duration of ischemia.

The increase in IR of GFAP found in the postresuscitation period may have been the result of activation of the synthesis of this protein. Many factors stimulating growth of astrocytes and raising their GFAP level are known [5, 8]. However, an increase in IR of GFAP is possible even without activation of its synthesis, when the mechanism of enhancement of immunoreactivity may be union of the glial filaments, creating an evident increase in their concentration, or the appearance of new, previously masked epitopes for union with the antibody in hypertrophied astrocytes [9]. However, whatever the causes giving rise to it, the increase in IR of GFAP is evidence of a change in the state of the astrocytes in the postresuscitation period. Another fact which seems extremely important is the appearance of GFAP-expressing astrocytes in the gray matter of the brain of rats surviving systemic circulatory arrest, which raises the question of the sources of origin of these new IR GFAP of the astrocytes. The results of investigations using a combination of immunohistochemical and autoradiographic methods [13] provide convincing proof that the increase in the number of reactive astrocytes in brain injury takes place, not as a result of proliferation of GFAP-containing astrocytes that are normally present, but through transformation of protoplasmic astrocytes, not previously expressing GFAP. The cause of conversion of normal astrocytes into the reactive form may be penetration of immunoglobulins from the blood into the brain tissue [6]. After systemic circulatory arrest in rats, injury to the blood-brain barrier takes place even in the early postresuscitation period, and it is manifested as extravasation of

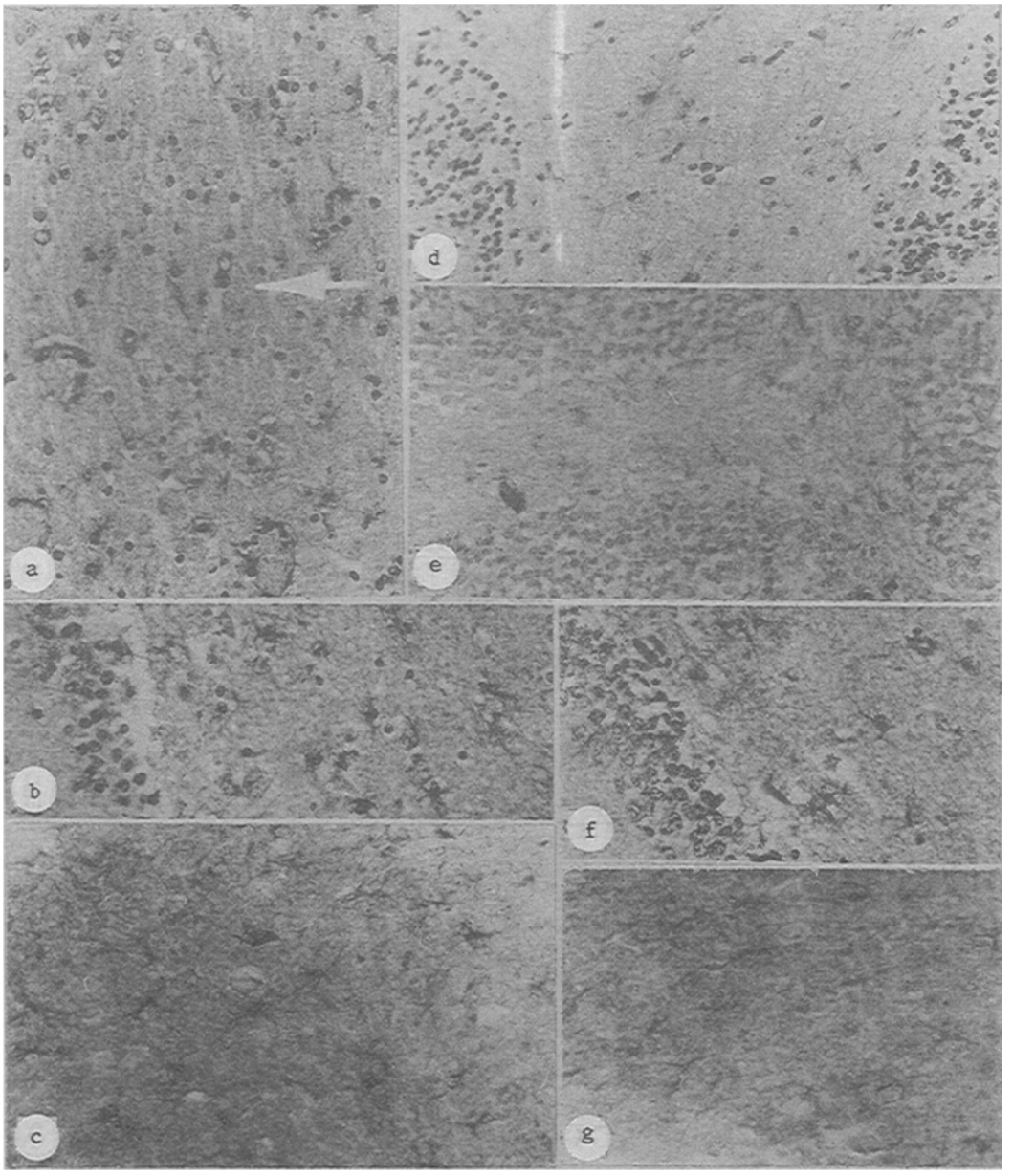

Fig. 1. GFAP-expressing astrocytes in different parts of brain of rats exposed to systemic circulatory arrest for 10 min: a) appearance of reactive glia in layer VI of sensomotor cortex. (Arrow indicates a stained astrocyte). PAP-hematoxylin, 100×; b, c) GFAP-positive astrocytes in cerebellum (c) and hippocampus (b) of rats 4 days after resuscitation. PAP-hematoxylin, 400×; d) weak immunoreactivity in astrocytes of white matter of cerebellum in intact rats. PAP-hematoxylin, 250×; e) increase compared with control in immunoreactivity in white matter of rat cerebellum 7 days after resuscitation. PAP-hematoxylin, 250×; f, 9) immunoreactive astrocytes in hippocampus (f) and cerebellum (g) of rats 7 days after resuscitation. PAP-hematoxylin, 400×.

blood proteins and infiltration of the brain tissue by them [14]. It can therefore be postulated that sudden changes of IR in astrocytes of the sensomotor cortex, hippocampus, and cerebellum of resuscitated animals are associated with transformation of astrocytes, caused by disturbances of permeability of the blood-brain barrier.

On the whole, correlation can be detected between the severity of the morphological changes in the brain neurons of resuscitated animals and the level of IR of GFAP. However, the localization of IR GFAP of the astrocytes by no means coincides with the regions of multiple changes in neurons but, on the contrary, immunoreactive

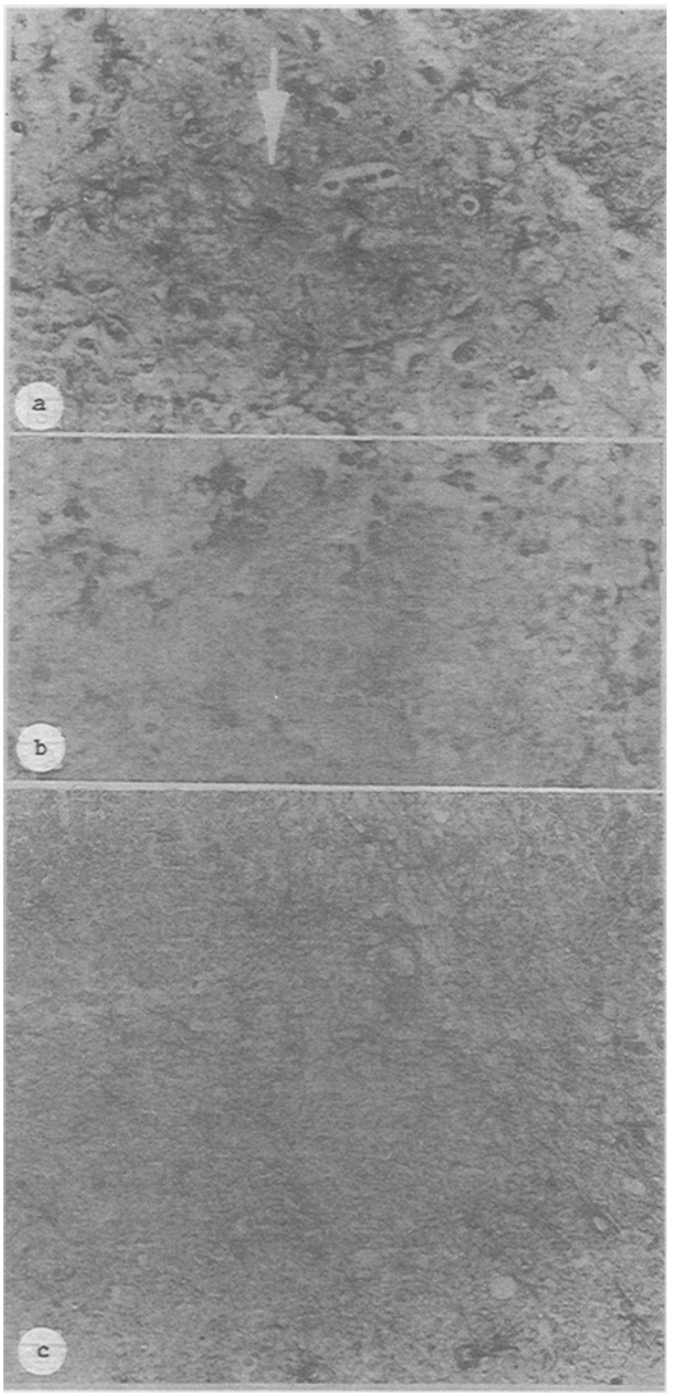

Fig. 2. Sharp increase in immunoreactivity of GFAP in astrocytes of hippocampus (a), cortex (b), and cerebellum (c) after systemic circulatory arrest for 15 min. PAP-hematoxylin, 400×.

astrocytes are only rarely found side by side with normal nerve cells. Similar results were obtained in a study of the motor cortex of neurological patients [10]. The absence of immunoreactive astrocytes in regions of maximal damage to neurons may be connected with the fact that the glia also is worst affected there, and as a result the GFAP disappear or their synthesis is disturbed. However, another possibility is that changes in neurons and glia may take place relatively independently of each other, as is shown, in particular, by the fivefold increase in expression of GFAP in progressive dementia before the development of any changes in neuronal structure [12]. After systemic circulatory

arrest expression of GFAP is found in astrocytes of the molecular layer of the cortex and sectors CA1-CA2 of the hippocampus, where no marked changes in the neurons were observed. In layers II-III of the cortex and in the layer of Purkinje cells in the cerebellum, where foci of disappearance of nerve cells and multiple degenerative changes in neurons are found, IR of GFAP of the glia was absent. These facts also are evidence in support of the view that an increase in IR of GFAP in the postresuscitation period cannot evidently be simply the response of astrocytes to neuronal damage, as some workers consider [15], but it is evidently the result of direct action of the damaging factors on the glia.

The results of this investigation thus revealed significant changes in the state of the astrocytes in different parts of the brain in the postresuscitation period and dependence of these changes on the severity of the responsible factors, the stage of the postresuscitation process, and the degree of restoration of the neurological status of the animals. The results point to the possibility of a change in the state of the astrocytes in the absence of any marked damage to neurons, and this points to a role of disturbances of glial homeostasis in the formation of postresuscitation brain pathology.

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