Bulletin LXXXVII de l'Academie Serbe des Sciences et des Arts — 1984
Classe des Sciences medicales № 13

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EFFECT OF INDOMETHACIN ON THE MORPHOLOGY OF THE BRAIN VASCULAR NETWORK IN THE POSTISCHEMIC PERIOD

Cerebral circulatory disturbances, varying in nature, intensity and duration have been shown to follow temporary brain ischemia [Waltz and Sund, 1967; Ames *et al.*, 1968; Crowell and Olsson, 1972; Mchedlishvili *et al.*, 1974; Kapuściński, 1974; Kapściński *et al.*, 1975; Mrsulja *et al.*, 1975; Kelly and Halsey, 1976; Fischer *et al.*, 1977; Crockard *et al.*, 1980]. They reflect on the state of vascular network of the brain and therefore can be visualized by morphological methods [Mossakowski, 1974]. It has been shown that in the most early postischemic stage the vascular abnormalities consist of generalized brain hyperemia, with a predominance of venous congestion, which increases in time. Morphological exponents of local ischemia appear simultaneously in the border-zones between areas vascularized by larger cerebral arteries. The most typical feature is characterized by the occurence of either small patches of ischemia spread widely throughout the grey matter structures (mostly in the cerebral cortex and thalami) or small foci of ischemia intermingled with those of hyperemia. These abnormalities show no relationship with cerebral angioarchitectonics and persist for the longest time of the recovery period [Mossakowski, 1978]. Their pathogenetic mechanism is not fully understood although numerous factors were taken into consideration to explain their appearance. Among others were prostaglandins synthetized locally in the brain [Mossakowski, Gadamski, 1978]. The increased level of prostaglandins in the brain resulting from its ischemia was demonstrated by several authors, among others by Ruszczewski [1977], Gaudet and Levine [1979], Iannotti *et al.* [1981] and Bhakoo *et al.* [1982]. Recently Furlow and Hallenbeck [1978] and Hal-

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lenbeck and Furlow [1979] documented the fact that premedication of temporary brain ischemia with indomethacin, a well-known inhibitor of prostaglandin synthesis, prevents or greatly reduces the postischemic brain circulatory disturbances.

This leads us to study the influence of the prostaglandin synthesis inhibitor on the morphology of the cerebral postischemic circulatory abnormalities.

Material and Methods

Experiments were performed on 4-month-old Mongolian gerbils of both sexes. Under light ether anesthesia the left carotid artery was ligated for a period of 15 min in both experimental and control animals. Based on the clinical criteria animals were divided into symptomatic and asymptomatic groups. Asymptomatic animals, showing no neurological abnormalities exept left-sided Horner's syndrome, were excluded from further studies. Both experimental and control animals were decapitated without anesthesia in groups of two or three immediately after release of the carotid artery ligation and then 15 min and 2, 6, 12, 24 and 48 hr following the end of brain ischemia.

All experimental animals, 45 min prior to carotid artery ligation were given an intraperitoneal injection of indomethacin (Merck-Sharp and Dohme, Res. Lab. USA), dissolved in Krebs-Ringer solution in a dosis of 10 mg/1 kg.* Those which survived longer than 6 hr were given the same dosis of the drug every subsequent 6 hrs. The control animals were treated in the same manner as experimental ones, but drug injections were substituted with those of Krebs-Ringer solution alone.

Sham-operated animals and those not subjected to any experimental procedure, both given and not given drug injections, served as additional controls.

The brain were fixed in neutral 10 percent formalin and then cut frontally into blocks on the level of the anterior portion of basal ganglia and rostrally at the height of fully developed thalami and hypocampal gyri. Frozen sections, stained by the Pickworth benzidine method were used for histological examination.

Results

In untreated animals arterial release brought about an almost complete blurring of normal angioarchitectonics of all examined

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structures in the ischemic brain hemisphere. Severe engorgement of almost all larger vessels, poor and uneven visualization of the capillary network and irregular distribution of greatly distended veins dominated the picture of the cerebral cortex, striatum and thalamus (Fig. 1). The white matter structures seemed to be less involved, hyperemic features were less evident here. Already at this stage of observations some well defined areas, located in certain regions of the neocortex and thalami, showed a great reduction of the capillaries filled with stained erythrocytes. On the basis of their topography they could correspond to the border-zones between the areas vascularized by larger brain arteries. 15 min later the general pattern of vascular abnormalities was practically the same, but features of hyperemia were more pronounced. So were poor visualization of the capillary network in the cerebral cortex and basal ganglia and unevennes of distribution of hyperemic areas. Foci of vascular engorgement were irregularly intermingled with those showing a reduced content of erythrocytes (Fig. 2). At 2nd h following carotid release the vascular abnormalities were most intensive. At this time severe vascular engorgement was limited mostly to larger veins. Poor filling of capillary network was most extensive. Even in greatly hyperemic zones, small patches of reduced blood supply were present (Fig. 3). The white matter structures showed evident features of venous hyperemia. Some normalization of the morphological picture of the cerebral vascular network started on the 6th hr following arterial release. Venous hyperemia, although present, was less intensive, capillaries were more evenly filled with stained erythrocytes. However patchy foci of ischemia widespread throughout the cerebral cortex, were still numerous (Fig. 4) and regular areas of reduced blood supply in the fields considered as border-zones in the neocortex and thalami were still present. Features of further normalization were observed on the 12th and 24th hrs of the recovery period. Venous hyperemia gradually decreased, as did the number of foci with reduced blood supply. The latter were noted even in animals sacrificed at 24 hrs. Full normalization was observed after the 48th hr.

In all animals the hemisphere contralateral to the ligated carotid artery revealed some venous hyperemia during the early stages of the recovery period. There were occasional foci of reduced blood supply within the cerebral cortex and basal ganglia.

The morphological picture of the cerebral vascular network in indomethacin-treated animals, prior to carotid artery ligation revealed most of the same abnormalities, which occurred in the untreated animals but also same essential differences. Features of severe brain hyperemia, with predominant, if not exclussive, engorgement of the larger veins were common to both groups of animals. In animals in which carotid artery ligation lasted 15 min severe venous hyperemia followed immediately arterial release. It increased

in its intensity and extension in 15 min, reaching maximal severity at 2 hr of the recovery period (Fig. 5). Still intensive at the 6th hr (Fig. 6) it gradually decreased during further stages of observation. Hyperemic features were not present at 24 hrs. Venous hyperemia, while present, involved all grey, and to a lesser degree white, structures of the brain hemisphere on the side of ligated carotid artery.

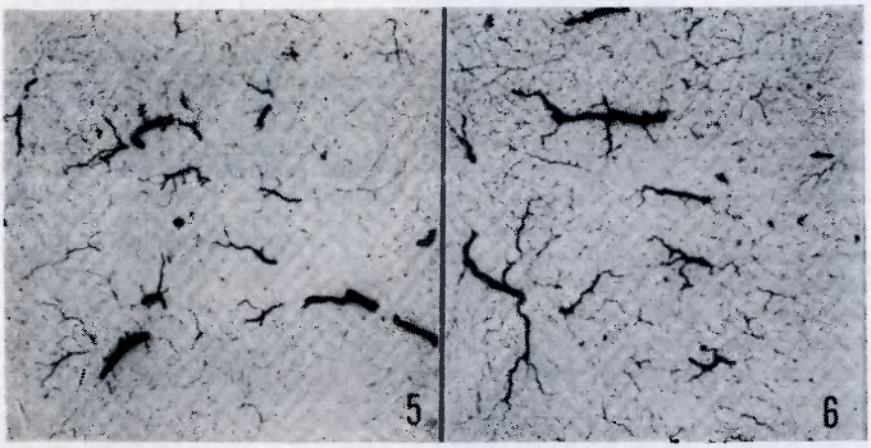

Fig. 5. Cerebral vascular network 15 min after release of the carotid artery ligation in an indomethacintreated animal. Severe engorgement of larger veins superimposed on normal capillary net. Pickworth benzidine. × 60.

Fig. 6. Cerebral vascular network 6 hrs after release of the carotid artery ligation in an indomethacintreated animal. Venous hyperemia on the background of normal capillary net. Pickworth benzidine. × 60.

The second phenomenon common to both treated and untreated animals, consisted of the occurrence of a great reduction of capillary network filled with stained erythrocytes in the border-zones between areas vascularized by larger cerebral arteries. This was present in the cerebral cortex in its region forming a border-line of middle and posterior cerebral arteries and in the thalami where borderline--zone between carotid and vertebral systems is located. Reduced blood supply to these areas appeared already at 15 min following brain ischemia and persisted till 6 hr (Fig. 7).

The most striking difference between indomethacin-treated and untreated groups of animals consisted in the fact that in the former capillary network in all cerebral structures was evenly visualized by stained erythrocytes in all examined stages of the recovery period (Figs 8—9). There was no irregular intermingling of the hyperemic areas with those showing features of ischemia. No small patches of focal ischemia spread throughout cerebral cortex were present at any time of the postischemic period.

Fig. 7. Cerebral vascular network 6 hrs after the release of the carotid artery ligation in an indomethacin-treated animal. Engorged veins on the background of the otherwise normal capillary net with two well-defined areas (arrows) of reduced capillary filling in the thalamus. Pickworth benzidine. × 60.

Fig. 8. Cerebral vascular network immediately after release of the carotid artery ligation in an indomethacintreated animal. Some hyperemic features with normal capillary filling in the cerebral cortex. × 60.

Fig. 9. Cerebral vascular network 2 hrs after release of the carotid artery ligation. Severe hyperemia with even capillary filling in the cerebral cortex white matter and thalamus. Pickworth benzidine. × 60.

Morphological abnormalities of the vascular network in the hemisphere contralateral to the ligated carotid artery were limited to some slight venous hyperemia of obviously lesser intensity than in the ischemic hemisphere. This was noted only in the earliest stages of the recovery period. The cerebral vascular network in sham-operated animals, both indomethacin-treated and untreated, did not show any differences when compared with that in animals which were not subjected to any experimental procedure.

Discussion

Our observations indicate, that indomethacin, administered 15 min prior to the carotid artery ligation influences the morphological picture of the cerebral vascular network during the postischemic recovery period. However the influence of the drug is limited to only one morphological component of the postischemic circulatory disturbances. Its application prevents the appearance of abnormalities in filling of capillaries with benzidine-stained erythrocytes. This finds morphological exponents in an even visualization of the cerebral capillary network and lack of patches of local ischemia spread throughout the neocortex and basal ganglia. This observation can be considered as a morphological ground for pathophysiological findings of Hallenbeck and Furlow [1979] who have shown that indomethacin administered prior to ischemia greatly enhanced cerebral blood flow and eliminated zones of focal flow impairment. Topographic convergence of the ischemic patches and disseminated neuronal lesions found in another model of cerebral ischemia [Mossakowski, Zelman, 1975] is strongly suggestive of the fact that the former can be responsible for the appearance of focal irreversible damage of the brain tissue. As patchy foci of ischemia spread throughout the cerebral cortex and basal ganglia are the most persistent features of postischemic circulatory disturbances, the indomethacin administration preventing them, shortens the duration of microcirculatory abnormalities during the recovery period.

The two remaining components of the circulatory disturbances, typical of the recovery period are resistant to indomethacin action. These are generalized venous hyperemia and impaired blood supply to the borderline-zones between watersheds of larger brain arteries. This may be indicative for differences in the pathogenetic mechanisms of particular components of postischemic circulatory abnormalities. It seems plausible to relate postischemic venous hyperemia with both local and systemic hemodynamic changes which follow brain ischemia. Local hemodynamic disturbances were demonstrated by Mchedlishvili *et al.* [1974], as being due to an impairment of pial arterial mechanisms regulating cortical blood supply. Bilateral distribution of the postischemic cerebral hyperemia, although

prevailing on the side of carotid ligation and evident features of impaired blood supply to borderline-zones indicate pathogenetic participation of the systemic hemodynamic abnormalities. This is supported in observations concerning the reduction of systemic blood pressure resulting from both uni- and bilateral carotid artery ligation in gerbils [Kapuściński, Mossakowski, 1981; Ito *et al.*, 1975].

Less unequivocal is the pathomechanism of focal reduction of the blood vessel filling, observed in brains of untreated animals. The same reduction in hypovolemic hypoxia has been considered as resulting from functional abnormalities of pial vessels found in this experimental model [Mchedlishvili, Baramidze, 1974] and is thought to express an impairment of the autoregulatory mechanisms. If one accepts this point of view in the case of unilateral carotid artery ligation, the question remains as to whether they are due to the influence of ischemia on nerve elements regulating cerebral blood flow [Rosendorf, 1974] or result from brain tissue metabolic changes. Observations of Mrsulja *et al.* [1976] concerning time relations between cerebral blood flow abnormalities and cerebral biogenic amine level in Mongolian gerbils with unilateral carotid artery ligation, strongly support the second supposition. The rise of brain prostaglandins level, found in the postischemic brain [Ruszczewski, 1977; Gaudet, Levine, 1979; Bhakoo *et al.*, 1982] opened the question of the pathogenic role of these vasoactive substances. Our present results, in which the inhibitor of prostaglandin synthesis was shown to prevent focal reduction of the blood supply to the brain during postischemic recovery may be considered as indicative of an action of prostaglandin-like substances in its appearance. Identical effects of aspirine, observed in another series of experiments (Mossakowski, Kwiatkowska-Patzer — unpublished data) also support this supposition. The exact mechanism of this action remains to be elucidated.

Acknowledgement

The authors are deeply indebted to Mrs Teresa Bok for her excellent technical assistance.

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