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COMPARATIVE MORPHOMETRIC ANALYSIS OF TERMINAL VASCULARIZATION OF HIPPOCAMPAL CA1 AND CA3 SECTORS IN MONGOLIAN GERBILS

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Comparative morphometric analysis of terminal vascularization (vessels with a diameter lower than 12.5 μm) in the ischemia-sensitive sector CA1 and the ischemia-resistant sector CA3 of Ammon's horn in Mongolian gerbils was performed. Basing on numerous computer-counted parameters characterizing the terminal vascular network in both hippocampal sectors and its relationship to the surrounding tissue, it was shown that a number of capillary vessels, their average diameter, and exchange and flow surfaces were to a statistically significant degree lower in the pyramidal layer of the CA1 sector as compared with those in CA3 sector. The number of pyramidal neurons in the pyramidal layer, counted per surface unit was in sector CA1 higher than in sector CA3. The obtained data indicate clearly an angioarchitectonically dependent lower microvascular capacity of sector CA1. However, these differences do not indicate *per se* a leading role of the vascular factor in the pathomechanism of selective vulnerability to ischemia. They may be a factor facilitating neuronal damage evoked by the excitotoxic action of glutamate, observed in CA1 sector as a result of forebrain ischemia.

Key words: *terminal vascularization, hippocampus, selective vulnerability, morphometry.*

According to the generelly accepted opinion, selective vulnerability of the pyramidal neurons in the CA1 hippocampal sector to ischemia is related with their specific synaptic organization, exposing them in this pathological condition to the excitotoxic action of glutamate (Collingridge et al. 1983; Rhotman 1984; Wieloch 1985; Rhotman, Olney 1986). Bioelectric hyperactivity resulting from this excitotoxic influence (Suzuki et al. 1983) is followed by intracellular calcium influx, which disturbs a number of essential cellular metabolic processes, leading to irreversible neuronal damage (Drejer et al. 1985; Neldrum et al. 1985; Choi 1987, 1988; Siesjö, Bengtsson 1989; Nakamura et al. 1993). Numerous biochemical, morphological and physiopathological studies carried out during the two latest decades supplied a great number of observations supporting this concept, among which these data should be mentioned, which indicate that postischemic selective damage of CA1 pyramidal neurons may be prevented by their preceding glutaminergic deafferentation or by the use of pharmacological blockers of specific NMDA receptors (Pulsinelli 1985; Simon et al. 1984).

In this context an old concept of Spielmeyer (1925) referring selective vulnerability of hippocampal formations to ischemia to their specific angioarchitectonics was dominated by hypotheses closer to the pathoclisis theory of C.O. Vogts (1922), understood in contemporary terms as functional and metabolic properties of the particular neuronal populations. However, several observations had been collected which indicate that the pathogenic role of the microvascular network in selectively vulnerable brain structures such as the hippocampus cannot be excluded.

The asymmetry of neuronal hippocampal lesions, concerning both their extension and intensity, in the experimental model of short-term ischemia of the forebrain in Mongolian gerbils can hardly be explained, basing exclusively on the excitotoxic concept of neuronal damage (Gadamski, Mossakowski 1992). It suggests some additional factor of factors, with high probability of vascular nature, being involved in the mechanism of the pathological events. Imdahl and Hossmann (1986) suggested that persistent postischemic hypoperfusion of the CA1 sector may play the role of an important pathological factor in the maturation of neuronal damage in this hippocampal area. On the other hand, the observations of Gadamski and Mossakowski (1992) showed that structural properties of the microvascular network in CA1 sector may facilitate hemodynamic abnormalities disturbing blood flow

restitution in this area in the postischemic period. Participation of the „vascular factor" in the development of pathological events in selectively vulnerable areas seems to be more probable, as different hippocampal regions, varying in their sensitivity to ischemic damage, were shown to be characterized by different angioarchitectonic properties (Coyle 1976, 1978; Wrzołkowa et al. 1992).

This inclined us to perform a comparative morphometric analysis of terminal circulation in two hippocampal regions: ischemia-sensitive sector CA1 and ischemia-resistant sector CA3. The same computer program was used as in a seriess of previous studies (Wrzołkowa et al. 1984, 1992; Kraszpulski, Wrzołkowa 1985, 1987).

Material and methods

The studies were performed on the brains of 9 adult Mongolian gerbils, weighing ca. 70-90 g, not submitted to any experimental procedures. The animals were anesthetized with pentobarbital sodium (Nembutal), given intraperitoneally in a dosis of 30 mg/1 kg of body weight. They were sacrificed by transcardiac perfusion (needle placed within aortal arch) with solution consisting of 2% paraformaldehyde and 1% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4. In each case ca. 150 ml of perfusate was used. Perfusion was performed under hydrostatic pressure of 40 mm Hg. The perfusion was preceded by a short rinsing of the vascular system with heparinized physiological saline solution.

The brains removed from the skull were immersed for 24 h in the above described perfusion solution. Then they were cut coronally into 1 mm slices. From slices containing dorsal hippocampus, tissue blocks from sectors CA1 and CA3 of Ammon's horn (Bregma 3.8-4.2 according to Paximos and Watson 1986) were taken with the use of a large lumbar punction needle, 1 mm in diameter. The tissue blocks were postfixed in buffered 3% osmium tetroxide and then dehydrated in graded solutions of alcohol. They were embedded in Epon 812. Embedding procedure during the first day was caried out at the temperature of 37°C and during the next two days – at 56°C.

The brain slices, from which tissue blocks were taken for Epon embedding, were processed to paraffin. Paraffin sections, stained with cresyl violet were used to control the structures from which material for electron microscopy was taken. Inproperly taken blocks were eliminated from further studies.

From Epon embedded blocks semithin sections (1.5 μm) were cut and stained with toluidine blue. They were photographed under $\times 125$ magnification. The final magnification of microphotographs used for morphometric analysis was $\times 1000$. Eighty one semithin sections were photographed. Microphotographs from 12 blocks of CA1 sector and 11 blocks from sector CA3 were selected for further studies.

On the microphotographs examined tissue fields and blood vessels were contoured and neurons in the pyramidal cell layers were counted. The microphotographs prepared in this way were then analysed semiautomatically with the use of a IBM/PC 386 computer equipped with a digitizer. Semithin sections were examined in a projecting Pictoval microscope (Carl Zeiss, Jena).

The above described analysis concerned the whole areas of CA1 and CA3 sectors as well as their separate layers – stratum oriens (A), stratum pyramidale (B) and stratum radiatum (C).

The following parameters were recorded:
- circumference and surface area of the examined hippocampal sectors;
- circumference and surface areas of their particular layers (A, B and C);
- circumference and surface areas of the larger blood vessels (diameter more than 12.5 μm);
- circumference and surface areas of the smaller blood vessels (diameter less than 12.5 μm);
- number of smaller vessels;
- number of pyramidal neurons in layers B.

The above data were used for establishing a number of computer-counted dependences, with application of appropriate mathematical formulas. These were as follows:
- relation of number of blood vessels in particular hippocampal layers and in the whole preparation to their total surface areas*,
- relation of number of blood vessels in layers A, B and C to the total surface areas of these sectors;
- relation of the total number of blood vessels to the number of pyramidal neurons in layer B;
- mean vascular surface in the whole sector and in its respective A, B, C layers;
- relation of number of pyramidal neurons to the surface area of B layer, and to the surface area of the whole preparation of the sector;
- linear density of vessels in the whole sector and in particular hippocampal layers examined;
- surface density of blood vessels in the whole sector and in its particular layers examined;
- volume density of blood vessels in the whole sector and in its particular layers examined.

For more legible presentation of the obtained numerical values all results were counted either per 1000 μm^2 or 1000 μm^3, respectively.

* All below presented values concern small vessels and surface areas reduced by the total surface areas of the larger vessels.

Stereometric transformations of the obtained data were performed according to the formulas described by Weible and Bolender (1973). Statistical analyses were done with non-parametric tests of Fisher-Pilman and Wilcoxon (Krauth 1988). Confidence level $\delta = 0.05$

Results

The obtained data clearly indicate significant differences between sectors CA1 and CA3 of the hippocampus as far as their terminal vascularization and cellular composition are concerned.

Not all of the examined features had the same distinguishing value. Statistical analysis limited the number of distinguishing features to 10 of the total 35 taken into consideration. Most of them concerned pyramidal cell layers. Table 1 compiles the features distinguishing two hippocampal sectors. In table 2 the mean values of distinguishing features are presented.

Table 1. Parameters distinguishing significantly sectors CA1 and CA3 of Ammon's horn

Examined feature	CA1 > CA3	CA3 > CA1
Number of vessels in pyramidal layer per preparation surface		+
Number of vessels in pyramidal layer per number of neurons in this layer		+
Average capillary surface in preparation		+
Average capillary surface in stratum oriens		+
Average capillary surface in stratum pyramidale		+
Vascular surface density in stratum pyramidale		+
Vascular volume density in stratum pyramidale		+
Surface area of stratum pyramidale		+
Number of cells in stratum pyramidale per surface in this layer	+	
Number of cells in stratum pyramidale per surface area of preparation		+

In general sector CA3 shows a much higher number of capillaries per surface unit than sector CA1 (0.53/0.18). The same difference concerns the number of vessels counted per number of pyramidal neurons in both sectors, amounting to 0.111 in sector CA3 and 0.045 in sector CA1.

Evaluation of capillary vessels parameters indicates much better vascularization of sector CA3. Average capillary surface in sector CA3 amounts to 10.15 μm^2, while in sector CA1 it is 8.32 μm^2. This difference is particularly visible in the pyramidal cell layer (9.23 μm^2 versus 6.70 μm^2) and in stratum oriens (10.93 μm^2 against 7.98 μm^2). The surface density, representing vascular exchange surface in the pyramidal cell layer in sector CA3, reaching 14.65 μm^2 is higher than in sector CA1, where it amounts to 11.93 μm^2. So is volume density characterizing flow volume which is 11.65 μm^3 and 8.25 μm^3 in sector CA3 and CA1, respectively.

Table 2. Mean values (x) of statistically significant parameters distinguishing sectors CA1 and CA3 of Ammon's horn

Examined feature	Measurement value	x	SD
Number of vessels in stratum pyramidale per area of preparation	n/1000 μm^2	0.18 0.53	0.10 0.14
Average capillary surface in:			
a. preparation	μm^2	8.32 10.15	2.22 2.80
b. stratum oriens	„	7.98 10.93	2.43 4.16
c. stratum pyramidale	„	6.70 9.23	4.91 2.18
Surface capillary density in stratum pyramidale	μm^2/1000 μm^3	11.93 14.65	6.28 3.60
Volume capillary density in stratum pyramidale	μm^3/100 μm^3	8.25 11.65	5.29 3.56
Number of vessels in stratum pyramidale per number of pyramidal neurons		0.045 0.111	0.026 0.033
Surface area of stratum pyramidale	1000 μm^2	4.17 12.04	1.73 3.85
Number of pyramidal neurons in stratum pyramidale:			
a. counted per surface area of stratum pyramidale	n/μm^2	29.44 11.82	4.44 2.32
b. counted per surface area of preparation	n/μm^2	4.02 4.83	0.64 0.87

* upper row gives concerning sector CA1, lower one those of sector CA3.

In addition to the above presented differences in the pattern of terminal vascularization the two examined sectors of Ammon's horn differ greatly in the number of pyramidal neurons. Sector CA1 contains more pyramidal cells per surface unit than sector CA3 (29.44 to 11.82), although the total area occupied by the pyramidal cell layer is in sector CA1 much smaller. Morphologically this finds its expression in almost invisible neuropil between neighboring neurons and hardly noticeable capillary vessels (Fig. 1), contrary to the broad intercellular neuropil accumulation and numerous capillaries in the pyramidal cell layer of sector CA3 (Fig. 2).

Despite the high proportion of pyramidal neurons per surface unit of the pyramidal layer of sector CA1, their number as counted for the surface of its three layers is lower than in sector CA3 (4.02/4.83).

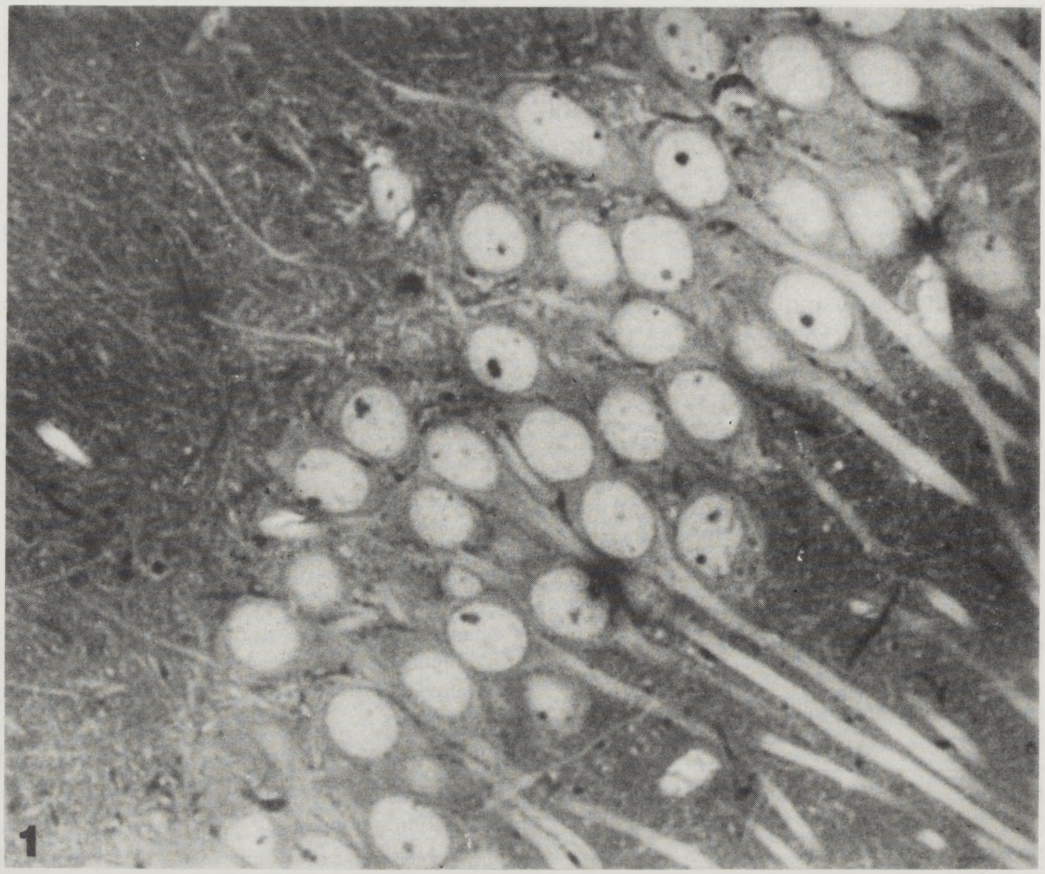

Fig 1. Sector CA1 of Ammon's horn. Densely packed pyramidal neurons, with hardly visible neuropil and capillaries between them. Semithin section, toluidine blue, × 790

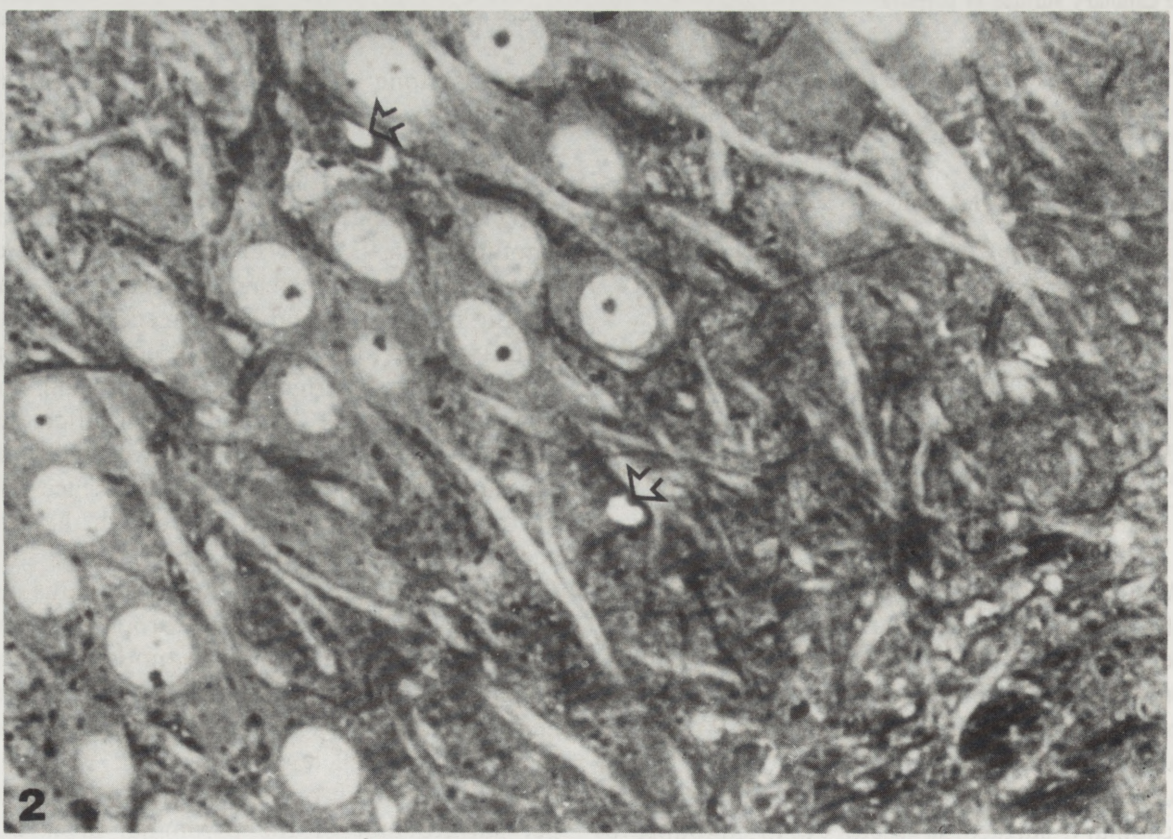

Fig 2. Sector CA3 of Ammon's horn. Less packed arrangement of pyramidal neurons with neuropil filling intercellular spaces. Capillary vessels are visible (arrows). Semithin section, toluidin blue, × 790

Discussion

Qualitative and quantitative differences of hippocampal vascularization as compared with other brain structures, first of all with cerebral cortex, were described in a number of papers (Spielmeyer, 1925; Coyle, 1976, 1978; Weiss et al. 1982; Schmidt-Kastner, Szymaś, 1990; Kumar et al. 1991). These peculiarities concern both larger arterial and venous vessels as well as microvascular structure. Less numerous and controversial are data concerning angioarchitectonic differences between hippocampal sectors, showing different sensitivity to ischemia. Coyle's studies (1976, 1978) indicated that the spacial arrangement of hippocampal vascularization might result in regional differences in blood supply. However, no quantification of this opinion was presented. Schmidt-Kastner and Szymaś (1990) while demonstrating, with the use of immunohistochemical methods, lesser capillary density in rat hippocampus as compared with that of neocortex, did not observe any differences in capillary supply between the ischemia-sensitive sector CA1 and ischemia-resistant CA3 sector. Similarly, Kumar et al. (1991) applying modern laser morphometry revealed in gerbils a much smaller number of perfused capillaries in the CA1 hippocampal sector than in neocortical formations, but did not find any statistically significant differences between CA1 and CA3 sectors. On this ground they denied the role of the vascular factor in pathogenesis of selective vulnerability to ischemia. Differences in capillary density between various parts of the hippocampus were recently described by Wrzołkowa et al. (1992). Imdahl and Hossmann (1986) found a slightly lower density of perfused capillaries in the hippocampal CA1 sector of gerbils than in sector CA3. This was considered to reflect a lower microcirculatory capacity of the CA1 area, resulting in its late postischemic hypoperfusion. Schmidt-Kastner and Freund (1991) in their extensive review on the mechanism of selective vulnerability, although critical to the concept of the pathogenic role of the vascular factor, conclude that the morphology of hippocampal vascularization indicates less secure blood supply to the CA1 sector than to the CA3 area and in the dentate gyrus.

Our studies aimed at comparison of terminal vascularization (blood vessels with diameter less than 12.5 μm) in sectors CA1 and CA3 of Ammon's horn. The analysis comprised both features characterized directly by morphometric measurements, and computer-counted indices, describing relations between the vascular network and surrounding brain tissue. The obtained data revealed a significant heterogeneity of both sectors. This was expressed by lower capillary density in sector CA1 as counted both per surface unit of the whole sector and of its pyramidal layer. Significantly lower was also the number of capillary vessels per number of pyramidal neurons. The characteristic feature of the CA1 sector consisted in smaller mean capillary surface as compared with sector CA3. Less favourable were computer-counted structural-functional parameters, characterizing the capillary network, such as exchange surface expressed by surface density, and flow volume expressed by volume density of capillaries. Differences in some parameters between particular cortical layers in the examined sectors are worth mentioning. For instance a greater average capillary surface in *stratum oriens* as compared with *stratum pyramidale*. Additionally, our analysis revealed that the number of pyramidal neurons per surface unit of the pyramidal layer was significantly higher in sector CA1 than in sector CA3. Our observations clearly indicate a lower microcirculatory capacity of the CA1 sector. Taking into account our earlier data (Gadamski, Mossakowski 1992) concerning the specificity of structural organization of vascular network within the pyramidal layer of CA1 sector and in its borderlines with neighboring layers (*stratum oriens* and *stratum radiatum*) it seems justified to assume that the angioarchitectonics of CA1 sector may facilitate the local blood flow disturbances observed in this area by Imdahl and Hossmann (1986). This seems particularly probable in conditions of incomplete cerebral ischemia (Kagström et al. 1983) and during late postischemic hypoperfusion (Blomqvist et al. 1984, Grogaard et al. 1989).

Differences in our observations as compared with those of Kumar et al. (1991) require a short comment, especially so as they concern the same experimental animals. It is possible that they reflect methodological differences. The studies of Kumar et al. (1991) are based on evaluation of the intravascular content of exogenous Evans blue. In this method only perfused vessels are visualized, while those which do not contain dye in their lumina (due for instance to compact accumulation of cellular blood elements) are invisible and not counted. Our studies, although statical, permit visualization (within limits of methodological error) of all or almost all vessels regardless whether they are or not perfused under *in situ* conditions. Additionally, the computer program applied permitted to establish that differences between the CA1 and CA3 sector concern not only the number of capillaries, but also such important parameters as vascular exchange surface and flow volume. Therefore, it seems that our observations present a real anatomical situation, characterizing the network of terminal blood vessels in CA1 sector. Under conditions of cerebral

ischemia this may be superimposed by a number of pathophysiological phenomena such as excitotoxic activity of glutamate with its functional and metabolic consequences (Olney 1988; Choi 1988; Nakamura et al. 1993), temporary reduction of inhibitory activity of GABA-ergic interneurons (Gajkowska et al. 1989), changes in perfusion pressure, both during and after ischemia (Kagstrom et al. 1983; Blomqvist et al. 1984; Grogaard et al. 1989), disturbances of coupling mechanisms, regulating blood flow and energy requirements (Hossmann, 1982) and perhaps disturbances of cellular energy metabolism, facilitating the excitotoxic action of glutamate (Novelli et al. 1988). Recent studies of Abe et al. (1993) indicate that these disorders are present not only during ischemia, but also in late postischemic periods.

It seems obvious that even a lower microcirculation capacity as observed in the CA1 hippocampal sector, does not necessarily indicate the leading role of the vascular factor in the pathomechanism of selective vulnerability to ischemia. However, coexistence of both factors – excitotoxic action of amino acid neurotransmitters and specific angioarchitectonic properties, may be responsible for the sequence of pathologic events in the hippocampal CA1 sector, resulting from cerebral ischemia.

Porównawcza analiza morfometryczna unaczynienia terminalnego okolic CA1 i CA3 rogu Amona u chomików mongolskich.

Streszczenie

Przeprowadzono porównawczą analizę morfometryczną sieci naczyń terminalnych (średnica naczyń mniejsza niż 12.5 μm) we wrażliwym na niedokrwienie sektorze CA1 i opornym na niedokrwienie sektorze CA3 hipokampa u chomika mongolskiego. W oparciu o ocenę licznych parametrów charakteryzujących sieć naczyń terminalnych w obu sektorach i jej relację do otaczającego podłoża tkankowego wykazano, że liczba naczyń włosowatych, ich wielkość oraz powierzchnia wymiany i przepływu są statystycznie znamiennie mniejsze w warstwie komórek piramidowych sektora CA1 niż w tej samej warstwie sektora CA3. Natomiast liczba neuronów piramidowych w warstwie piramidowej w sektorze CA1 jest znacznie większa w przeliczeniu na jednostkę powierzchni niż w sektorze CA3. Uzyskane dane wskazują na angioarchitektonicznie uwarunkowaną mniejszą wydolność mikrokrążenia w polu CA1 w porównaniu z polem CA3. Odrębności te nie przesądzają jednoznacznie o decydującej roli czynnika naczyniowego w patogenezie wybiórczej wrażliwości na niedokrwienie. Mogą one być jednak czynnikiem pogłębiającym uszkodzenia neuronalne w sektorze CA1 wywołane przez ekscytotoksyczne działanie glutaminianu.

References

1. Abe K, Kawagoe J, Lee T-H, Aoki M, Kogure K: Disturbances of mitochondrial DNA expression in gerbil hippocampus after transient forebrain ischemia. Molec Brain Res, 1993, 19, 69-75.
2. Blomqvist P, Lindvall O, Wieloch T: Delayed postischemic hypoperfusion: evidence against involvement of the noradrenergic locus coeruleus system. J cerebr Blood Flow Metab. 1984, 4, 425-429.
3. Choi DW: Calcium-mediated neurotoxicity: relationship to specific channel types and role of ischemic damage. Trends Neurosci, 1987, 11, 465-469.
4. Choi DW: Glutamate neurotoxicity and diseases of the central nervous system. Neuron, 1988, 1, 623-634.
5. Collingridge GL, Kehl SJ, Loo R, McLennan H: Effects of kainic acid and other amino acids on synaptic excitation of rat hippocampus. 1. Extracellular analysis. Exp Brain Res, 1983, 52, 170-178.
6. Coyle P: Vascular patterns of the rat hippocampal formation. Exp Neurol, 1976, 52, 447-458.
7. Coyle P: Spacial features of the rat hippocampal vascular system. Exp Neurol, 1978, 58, 549-561.
8. Drejer J, Benveniste H, Diemer NH, Schousboe A: Cellular origin of ischemia induced glutamate release from brain tissue in vivo and in vitro. J Neurochem, 1985, 45, 145-151.
9. Gadamski R, Mossakowski MJ: Asymmetric damage of the CA1 sector of Ammon's horn after short-term forebrain ischemia in Mongolian gerbils. Neuropatol Pol, 1992, 30, 209-219.
10. Gajkowska B, Gadamski R, Mossakowski MJ: Influence of short-term ischemia on the ultrastructure of hippocampal gyrus in Mongolian gerbils. II Electron microscopic picture of synapses in early postischemic period (In Polish). Neuropatol Pol, 1989, 27, 339-366.
11. Grogaard B, Schürer L, Gerdin B, Arforst KE: Delayed hypoperfusion after incomplete forebrain ischemia in the rat. The role of polymorphonuclear leukocytes. J cerebr Blood Flow Metab. 1989, 9, 500-505.
12. Hossmann K-A: Treatment of experimental ischemia. J cerebr Blood Flow Metab, 1982, 2, 275-297.
13. Imdahl A, Hossmann K-A: Morphometric evaluation of capillary perfusion in selectively vulnerable areas of gerbil brain. Brain Res, 1986, 239, 57-69.
14. Kagström E, Smith M-L, Siesjö BK: Recirculation in the rat brain following incomplete ischemia. J cerebr Blood Flow Metab, 1983, 3, 183-192.
15. Kraszpulski M, Wrzołkowa T: Morphometric evaluation of terminal vessels. Methodological studies. Neuropatol Pol, 1986, 24, 491-498.
16. Kraszpulski M, Wrzołkowa T: Blood-brain exchange in various regions of the limbic system. Morphometric studies. Neuropatol Pol, 1987, 25, 265-271.
17. Krauth J: Distribution-free statistics; an application oriented approach. Elsevier, 1988 pp 164-176.
18. Kumar K, Goodrich J, Marcoux F: Comparison of vascular perfusion in ischemia-sensitive and ischemia-resistant regions of gerbil brain by an automated laser cytometric device: a preliminary study. J Neurosci Methods 1991, 39, 1-8.
19. Meldrum B, Evanös M, Griffith T, Simon R: Ischemic brain damage: the role of excitatory activity and calcium entry. Br J Anaesthesiol, 1985, 57, 44-46.
20. Nakamura K, Hatakeyama T, Furuta S, Sakaki S: The role of early Ca^{2+} influx in the pathogenesis of delayed neuronal death after brief forebrain ischemia in gerbils. Brain Res, 1993, 613, 181-192.
21. Novelli A, Reilly JA, Lysko PG, Henneberry RC: Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res, 1988, 451, 205-212.
22. Olney JW: Endogenous excitotoxins and neuropathological disorders. In: Excitatory amino acids in Health and Disease. Ed.: D Lodge. John Wiley, London, 1988, pp 337-351.

23. Paximos G, Watson C: The rat brain in stereotaxic coordinates. Academic Press, New York, 1986, pp 32-37.
24. Pulsinelli WA: Deafferentation of hippocampus protects CA1 pyramidal neurons against ischemic injury. Stroke, 1985, 16, 144-146.
25. Rhotman SM: Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. J Neurosci, 1984, 4, 1884-1891.
26. Rothman SM, Olney JW: Glutamate and the pathology of hypoxic ischemic brain damage. Ann Neurol, 1986, 19, 105-111.
27. Schmidt-Kastner R, Szymaś J: Immunohistochemistry of glial fibrillary acid protein, vimentin and S-100 protein for study of astrocytes in hippocampus of rat. J chem Neuroanat, 1990, 3, 179-192.
28. Schmidt-Kastner R, Freund TF: Selective vulnerability of the hippocampus in brain ischemia. Neurosci, 1991, 40, 599-636.
29. Siesjö BK, Bengtsson F: Calcium fluxes, calcium antagonists and calcium-related pathology in brain ischemia and spreading depression: a unifying hypothesis. J cerebr Blood Flow Metab, 1988, 9, 127-140.
30. Simon RP, Swan JH, Griffiths BS: Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science, 1984, 226, 850-852.
31. Spielmeyer W: Zur Pathogenese ortlich elektiver Gehirnveranderungen. Z Ges Neurol Psychiatr, 1925, 99, 756-776.
32. Suzuki R, Yamaguchi T, Choh-Li, Klatzo J: The effect of 5-minute ischemia in Mongolian gerbils. II Changes in spontaneous neuronal activity in cerebral cortex and CA1 sector of hippocampus. Acta Neuropathol (Berl), 1983, 60, 217-222.
33. Vogt C, Vogt O: Der Begriff der Pathoklise. J Psychol Neurol, 1925, 31, 245-262.
34. Weibel ER, Bolender RP: Stereological techniques for electron microscopic morphometry. Basic stereological methods. In: Principles and techniques of electron microscopy Ed: MA Hayat. Van Nostrand Reinhold, New York, 1973, vol 3, pp 244-277.
35. Weiss HR, Buchweitz E, Murtha TJ, Auletta M: Quantitative regional determination of morphometric indices of the total and perfused capillary network in rat brain. Circ Res, 1982, 51, 494-503.
36. Wieloch T: Neurochemical correlates to selective neuronal vulnerability. Prog Brain Res, 1985, 63, 69-85.
37. Wrzołkowa T, Cofta T, Łukaszczyk J: Capillary blood vessels of the brain. I. Vascularization density in various parts of the cat and rat cerebral cortex. Neuropatol Pol, 1984, 22, 85-96.
38. Wrzołkowa T, Łukaszczyk J, Rudzińska-Kisiel T, Kraszpulski M: Terminal vessels of dentate gyrus in chronically alcohol-intoxicated rats. Alcohol, 1992, 9, 271-274.

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