Changes in the distribution of sugar receptors in the brain of Mongolian hamster induced by 5-minute-long ischaemia

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The aim of the present study was to investigate the changes in the localisation of glycoconjugates caused by 5-minute-long ischaemia as a consequence of bilateral ligation of common carotid arteries. Histochemical evaluation was performed using specific lectins that recognize the following sugar residues: 1) α-D-galactosyl and N-acetylgalactosaminyl (Bandeiraea simplicifolia agglutinin, BS-I), 2) N-acetylβ-D-galactosaminyl (Ricinus communis, agglutinin, RCA-60), 3) N-acetylα-D-galactosaminyl (Helix pomatia agglutinin, HPA), 4) α-D-mannosyl and α-D-glucosyl (Concanavalin A), 5) α-D-fucosyl (Tetragonolobus purpureas agglutinin, TG).

It was found that the first two lectins (Bandeiraea simplicifolia and Ricinus communis) bound selectively to endothelium, while two others (Helix pomatia and Concanavalin A) showed affinity predominantly to cellular compartments of the brain, like neurons and glia, but also to neuropil and the white matter. Tetragonolobus purpureas agglutinin did not stain any brain structures. Significant changes in the arrangement of examined glycoconjugates as a result of ischaemia were observed. First, the intensity of binding of BS-I and RCA-60 to their complementary residues was increased in the capillary network as compared with control animals. Glycoconjugates recognised by these lectins were also found in reactive astrocytes in various brain structures. Second, the number of sugar receptors visualised by Con A and HPA was enhanced in the majority of cellular compartments of the brain and, though to a lesser extent, in terminal capillary network, especially in the neocortex, while in control animals these sugar receptors were detected in brain microvessels. Redistribution of particular sugar residues after ischaemia suggests that functional changes associated with this type of injury might take place in various CNS structures.

key words: lectins, brain ischaemia, sugar receptors
sugar residues on the surface of these cells using lectins, interesting per se, suggest also abnormalities of the BBB system [17, 18, 28, 32]. Such differences in the localization of some sugar residues in the vascular walls in rats that survived experimentally-induced clinical death and other ischaemic incidents have been observed by us earlier [20, 22, 23, 25]. Thus, it was of interest to characterise the putative rearrangement and relocation of sugar residues in oligosaccharide chain, using different animal model of ischaemia with special regard to those brain structures that have not been previously examined.

**MATERIAL AND METHODS**

The experiments were carried out on fifteen adult Mongolian hamsters. Brain ischaemia had been caused by bilateral ligation of common carotid arteries for a period of 5 min. The experimental animals survived 6 (4 animals), 12 (3 animals), 24 (4 animals) and 48 (4 animals) hours (hrs) after cerebral ischaemia. Then, they were sacrificed under ether anaesthesia by transcardiac perfusion with fixative solution consisting of 4% formalin in PBS (pH 7.4), preceded by short perfusion of 0.9% saline solution. After perfusion, the brains were removed from the skull, immersed in the abovementioned fixative for an additional 48 hrs. Tissue blocks were cut coronally at the level of nucleus caudatus/putamen, dehydrated in alcohol and embedded in paraffin. Then, 20 μm sections were deparaffinised and submitted to histochemical procedure (methodological details in the paper by Szumańska et al. [26, 27]) in order to reveal the following sugar residues:

1. α-D-galactosyl and N-acetyl-α-D-galactopyranoside (using Bandeiraea simplicifolia agglutinin, BS-I)
2. N-acetyl-β-D-galactosyl (using Ricinus communis agglutinin, RCA-60)
3. N-acetyl-β-D-galactosaminyl (using Helix pomatia agglutinin, HPA)
4. α-D-mannosyl and α-D-glucosyl (using Concanavalin A, Con A)
5. α-L-fucosyl (using Tetragonolobus purpureas agglutinin, TG).

Localisation of those glycoconjugates was studied at the level of telencephalon in the neocortex, nucleus caudatus, area septi, plexus choroideus and corpus callosum. The incubation media contained biotinylated lectins in the final concentration of 10 or 20 μg/ml. Then, the sections were incubated using the Vectastain ABC kit, which contained Reagent A (avidin DH) and Reagent B (biotinylated horseradish peroxidase H). The final reaction product was visualised by incubation in the solution of 3,3'-diaminobenzidine (DAB). The sections were counterstained with 1% cresyl violet, dehydrated in ethanol and embedded in Permount.

Specificity of histochemical reaction was controlled by preincubation of sections with 0.2 M solution of the corresponding hapten sugar and by incubation in a solution of PBS which did not contain a lectin.

**RESULTS**

**Sugar receptors visualised by BS-I**

In control animals, sugar residues α-D-gal and α-D-gal-Nac recognised by BS-I were present in single microblood vessels of the cortex (especially in its deeper layers), nucleus caudatus and area septi (mainly in nucleus medialis septi) (Fig. 1) as well as in stroma of connective tissue in plexus choroideus with negative cells in it. Weaker positive reaction was observed in fibres of corpus callosum and its capillaries, while diffuse staining was visible in areas surrounding fibrae capsulae internae of nucleus caudatus. BS-I also labelled the above-mentioned residues located subependymally in neuropil of lateral ventricles, whereas ependymal cells as such remained unstained.

In the group of experimental animals that survived 6 hrs after ischaemia, the intensity of staining was enhanced in capillaries of nucleus caudatus and area septi but it was reduced in microvessels in the neocortex. It has to be noted that an additional punctate reaction with the lectin appeared in close vicinity to BS-I-positive microvessels with swollen tissue around (Fig. 2). At this time-point, single cells reacting with BS-I were irregularly scattered between the vessels (Fig. 2). Twelve hours after ischaemia, positive binding of the lectin to neocortical capillaries reappeared. These capillaries as well as reactive microvessels in other brain structures of animals that were sacrificed 6 and 12 hrs after the insult seemed to be hypertrophic (swollen) (Fig. 3). At 24 hrs after ischaemia, the number of sugar residues recognised by the lectin was significantly reduced, both in the vessel network and other CNS structures, e.g., fibres of corpus callosum. However, in the group of animals that survived 48 hrs after ischaemia, the persistence of binding to glycoconjugates in capillaries of nucleus caudatus was found. At the same time, the appearance of reaction in numerous cells, including microglial cells, not exclusively associated with the vessel network, was observed in nucleus caudatus as well as, though to a lesser extent, in area septi (Fig. 4).

**Sugar receptors visualised by RCA-60**

Using RCA-60, the type of Nac-D-gal residues was detected in the majority of CNS structures of control
Positive reaction was visible in neuropil of the grey and white matter of the brain with slightly higher expression noticed in subependymal zones of lateral ventricles. Histochemical staining was even stronger in the capillary network (Fig. 5), especially in the neocortex, but also in area septi.

In the group of experimental animals that survived 6 and 12 hrs after ischaemia, there was a remarkable
Figure 5. Control gerbil. Neocortex. Positive reaction with RCA-60 showing N-acetyl-β-D-galactosaminyl residues visible in the neuropil of grey matter. The staining is stronger in the vascular network, × 200.

Figure 6. Experimental gerbil, 12 hours of survival after 5-minute-long ischaemia. Decrease intensity of staining with RCA-60 in the neocortical neuropil and in the microvessels (when compared with Fig. 5), × 200.

decrease in the number of glycoconjugates labelled with RCA-60, both in microvascular walls and in neuropil, especially well visible in all neocortical layers (Fig. 6). Between 24 and 48 hrs after ischaemia, relatively abundant binding of RCA-60 to sugar residues was found in brain vessels (mainly in capillaries of the neocortex and area septi). In addition, in the group of animals that survived 48 but not 24 hrs after ischaemia, the appearance of cells labelled with RCA-60, predominantly in II and IV neocortical layers, were detected. Some of them were localized in close vicinity to vessels (Fig. 7). RCA-60-positive cells were also present in various nuclei of area septi, e.g., nucleus lateralis septi.

Sugar receptors visualised by HPA

In the brains of control animals, strong and punctate reaction with HPA indicating the presence of N-ac-D-gal residues was found exclusively in bodies and processes of neurons scattered within III and V neocortical layers (Fig. 8). Weaker, diffuse staining was associated with fibres of white matter in corpus callosum as well as in internal capsule of nucleus caudatus. Other components of the grey matter like neuropil, ventricle ependyma, plexus choroideus and nonneural cell populations were not negative. However, terminal endings of capillaries in area septi were labelled with HPA.

In the group of animals that were sacrificed 6 hrs after ischaemia, histochemical reaction was significantly upregulated in neuronal cells in the neocortex as compared with controls. Both the number of labelled neurons and the intensity of staining per cell were increased, suggesting that the level of incorporation of sugar residues recognised by the lectin into cellular structures was high at this postischaemic time-point (Fig. 9). There was a faint staining of single neurons at the border of the grey and white matter in the neocortex and stronger, punctate labelling of large neuronal cells in area septi (Fig. 10). Also, progression of histochemical reaction in the fibres of the white matter was noticed. At longer postischaemic time-points (12 and 24 hrs), lectin stainability temporarily decreased in all the above-mentioned brain structures but 48 hrs after ischaemia histochemical reaction was again significantly enhanced in these areas (Fig. 11). At the latest time-point studied (48 hrs), the increased staining appeared also in terminal capillary network in the neocortex. The latter effect was undetectable, either in control or in other experimental groups of animals.
Sugar residues visualised by Con A

Two types of sugar residues, \( \alpha\)-D-man and \( \alpha\)-D-gluc, recognised by Con A seemed to be associated predominantly with neuronal cytoplasm and, to a lesser extent, with neuropil in the brain of control Mongolian hamster. In the neocortex, the strongest labelling was observed in the cells at the border of the grey and white matter, i.e., fibro-protoplasmic astrocytes (Fig. 12). Large neu-
rons of nucleus caudatus and area septi as well as ependymal cells within ventricles of plexus choroideus and oligodendrocytes in corpus callosum were also Con A-positive. Microvessels in all described CNS structures remained unstained. However, the expression of glyco-conjugates labelled with the lectin was noticed in larger vessels.

At 24 and 48 hrs after ischaemia, there was an increase in staining of sugar residues reactive with Con A in the brain cells, both in neurons of the neocortex and area septi and glial cells in the white matter. What is more, sugar receptors recognised by the lectin were also visible in terminal network of microvessels — arterioles and venules — in the cortex as well as in area septi and nucleus caudatus (Fig. 13). Regarding the latter structure, its large neurons were also reactive (Fig. 14).

**DISCUSSION**

The study of the presented investigation demonstrated changes in the staining intensity and localisation of particular sugar residues recognised by specific lectins as a result of 5-minute-long ischaemia.

While there is considerable literature concerning disturbances of neuronal brain damage, vascular permeability and glial proliferation [1, 6, 10, 15] relatively little is known about lectin binding in particular structures of the CNS in pathological conditions, although our earlier data, supported by that of others, showed that blood-brain barrier disturbances in many models of experimental brain damage are accompanied by translocation of some glycoconjugates in particular areas and structures of the brain [4, 5, 8, 20, 22, 23, 26–29, 33, 34]. Additionally, we would like to draw attention to the fact that in the majority of the above-mentioned experimental animal models, which led to metabolic disturbances in BBB permeability, no evident morphological changes were observed. Thus, the existence of many types of sugar receptors on the surface of endothelial cells of vascular network as well as in neuronal and glial cells in the CNS has already been recognised and is well documented in literature, especially that published one or two decades ago but also nowadays [14, 17, 22, 26, 27, 32]. In these reports, it has been demonstrated that the intensity of binding of particular lectins to sugar residues in membranous structures and changes in their arrangement in the oligosaccharide chain are dependent on the metabolic state of the tissue and are different under various pathological conditions considering the intensity of damage and complexity of brain structure. As already mentioned, dysfunctions of the BBB system, resulting from a variety of pathological conditions including ischaemia, are important and frequently observed disturbances within the CNS. So far, the data regarding the cytochemical evaluation of the CNS abnormalities are limited, although changes in the permeability of terminal capillary network have been

**Figure 11.** Experimental gerbil, 6 hours of survival after 5-minute-long ischaemia. The strongest labelling of HPA in III neocortical layer. Positive HPA — binding in terminal network of microvessels (arrows), × 400.

**Figure 12.** Control gerbil. Strong Con A binding to cytoplasm of neocortical neurons showing $\alpha$-D-mannosyl and $\alpha$-D-glucosyl residues, × 400.
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Consequently, evidence has been accumulated indicating that sugar residues in the oligosaccharide chain, essential components of microvasculature, for the detection of which some lectins have been applied [20, 22, 23, 28, 30, 31], are also changed.

In this paper, results from studies performed on animals that survived a short period of time after ischaemia, i.e., 6 to 48 hrs, are presented. It was of interest to investigate whether and how far the distribution of sugar receptors localised to cell membranes and recognised by specific lectins proceeds in time morphological changes in the CNS. Our observations indicate that there were essential differences in the localization of examined glycoconjugates in endothelial cells of blood vessels as well as in neuronal and glial cells in the brain of Mongolian hamster after 5-minute-long ischaemia and that of control animal. Changes in binding of most of the lectins used in this study proceeds in time morphological changes in the CNS. However, the brains of both control and experimental groups of animals remained unstained when Tetragonolobus purpureas agglutinin recognising α-L-fucosyl type of residues was applied. Interestingly, this lectin revealed its complementary sugar residues in the walls of blood vessels and amyloid plaques in the brains of scrapie-infected mice [26], suggesting that the effect might be species- and tissue-specific.

Sugar receptors of α-D-galactose and acetylgalactosamine visualised by BS-I were observed in control animals, in capillaries of the majority of brain structures, in neuropil, but only in restricted areas, and in plexus choroideus. Weaker staining was associated with fibres of the white matter. Distribution and expression of glycoconjugates changed remarkably, depending on time of survival after ischaemia. For instance, the intensity of binding of BS-I to its target receptors was reduced as early as 6–12 hrs after ischaemia, while at longer survival time (48 hrs) it was significantly enhanced in both capillaries and numerous cells, including microglia. We obtained similar results regarding the distribution of receptors labelled by BS-I in the vascular network earlier in the brain of control rats. It has to be noted, however, that in those animals, after experimental post-resuscitation syndrome, the disappearance of BS-I receptors in practically the whole capillary network appeared already 3 hrs after resuscitation. Endothelial cells were also swollen and tissue spongy degeneration was visible [20]. However, Marioka et al. [9] observed an increase in staining of microglial cells using BS-I agglutinin as early as 20 min after brain ischaemia but they found a stronger reaction 4–5 days after reperfusion. Similarly, Streit and Kreutzberg [19] suggested that maximum labeling of the CNS structures using this lectin is around 3 or 4 weeks after injury. In this paper, we examined brains of hamsters that survived only 6 to 48 hrs and only in some restricted brain areas.

Figure 13. Experimental gerbil. An increase in staining of sugar residues reactive with Con A in neocortical neurons after 48 hours of survival of 5-minute-long ischaemia. Positive staining visible also in some microvessels (arrow). × 400.

Figure 14. Experimental gerbil, 48 hours of survival after 5-minute-long Ischaemia. Nucleus caudatus. Strong Con A labelling in large neurons and weak reactivity in some microvessels, × 400.
In control animals, N-ac-gal type of residues recognised by Ricinus communis (RCA-60) was found predominantly in microblood vessels and neuropil. Glycoconjugates stained by this lectin disappeared completely but temporarily in the brains of experimental animals. This effect was observed up to 12 hrs after the insult but at 48 hrs after ischaemia positive reaction reappeared. At that time almost all vessels forming the vascular network revealed the existence of the receptors recognised by RCA-60 and as well in some cells lying in the neocortex and area septi. We observed an evident decrease in the staining intensity but with RCA-120 in similar brain structures as a result of 3- and 4-minute-long ischaemia [23] and after experimental post-resuscitation syndrome [20]. Vorbrodt [28, 35] also published changes in the distribution of endothelial surface glycoconjugates associated with altered permeability of brain microblood vessels. Using RCA-1-gold complex, Vorbrodt [30] concluded that β-D-galactosyl residues, which in the control brain are relatively uniformly distributed on both luminal and abluminal plasmamembranes of the endothelial cells, play some special role in the function of these cells. The high concentration of these residues in brain microvessels suggests their engagement in intercellular recognition, signal transfer, transcytosis and in intercellular transport of proteins. He found as well that these residues were drastically reduced in pathology of the brain.

Two other glycoconjugates, such as N-acetyl-D-galactosaminyl residues, which are specifically recognised by HPA and α-D-mannosyl and α-D-glucosyl recognised by Con A, are known for their strong granular binding specificity to big neurons in some areas of normal brain.

Concanavalin A agglutinin besides neurons binds also more diffusely and weakly to astrocytes and oligodendroglia. The intensive staining reaction also appeared in ependymal cells of ventricles and epithelial ones of choroid plexus. Similar observations have been made by other authors [8] and by us in an earlier paper [23] in the group of experimental animals which survived 24 and 48 hrs after ischaemia we observed a moderate increase in the staining intensity with Con A agglutinin, which indicates the appearance of α-D-mannose and α-D-glucose receptors not only in neurons of neocortex, area septi, nucleus caudatus, glial cells of the white matter but also in microblood vessel wall network. The explanation of this phenomenon is not simple and contradicts the histochemical observation made by us after 3- and 4-minute-long ischaemia [23]. But in the brain of scrapie-infected mice [26] the wall of microblood vessels showed some positive although rather weak reaction with Con A agglutinin. According to Vorbrodt’s EM-observation [30], the residues of glucosyl and mannosyl are concentrated also on abluminal site of the microvascular wall, mainly in basal lamina. Such a result can be explained by the fact that in some pathological conditions not only sugar specificity but also conformation features and position in oligosaccharide chain have an influence on the localization of some sugar residues.

HPA binding to N-acetyl-D-galactosamine residues was limited in control brains to single, large neocortical neurons and their processes in layers III and V. In experimental animals as soon as 6 hrs after 5-minute-long ischaemia, an increase in HPA reaction in these cells was manifested by an increase in the number of stained neocortical neurons and by the accumulation of the reaction in branched dendrites. Additionally, positive HPA large neurons appeared in this survival group in area septi. At 12–24 hrs after ischaemia, reaction with this lectin slightly decreased but increased significantly again at 48 hrs after ischaemia. We published similar observations as a result of thioacetamide-induced hepatic failure in the rat brain [21].

The picture of the distribution of sugar receptors in the walls of brain vessels in the groups of animals that survived 5-minute-long ischaemia was different from the control one. The increase in binding of BS-I and RCA-60 as well as the appearance of labelling with HPA and Con A were observed in those brain structures where receptors were undetectable in control animals, especially 48 hrs after ischaemia. Changes in labelling of luminal and abluminal plasmamembranes of vascular endothelial cells with the use of Con A as a consequence of damage to BBB were observed also by other authors [31. 33, 36]. Similar effects in ultrastructural studies on animals with severe damage to blood-brain barrier (BBB) in which sugar receptors translocated from luminal into abluminal plasmamembrane of endothelium by means of pinocytic vesicles, invaginations and tight junctions were described by Williamson et al. [37]. These changes suggest the rearrangement of terminal positions of particular sugar residues in glycoprotein or glycolipid chain induced under pathological conditions. For instance, ultracytochemical studies [33] showed translocation of entire segments of luminal plasmamembrane of endothelial cells with which receptors recognised by Con A were associated, into pinocytic vesicles, vacuoles, and even abluminal plasmamembrane of vascular endothelium. Thus, it is reasonable to assume that sugar receptors of glycoconjugates are involved in the active transport after barrier mechanisms were disturbed.
The present study revealed that ischaemia produces also pronounced changes in the distribution and intensity of binding to various lectins in examined brain structures. Too little is known about the precise function of the sugar residues to associate them with specific brain dysfunction but the result manifested here shows significant rearrangements of monosaccharide residues in the CNS areas and their particular structures after ischaemia.

REFERENCES


