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Alteration of water-soluble S-100 protein content in microembolized rat brain.

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Abstract

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ALTERATION OF WATER-SOLUBLE S-100 PROTEIN CONTENT IN MICROEMBOLIZED RAT BRAIN

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Abstract. The amount of S-100 protein in rat brain embolized with carbon microspheres decreased in parallel with the development of cerebral edema as judged by water content, recovering to the normal range by 24 h after embolization. These results suggest the participation of S-100 protein in the permeability characteristics of nervous system capillaries known as the blood-brain barrier.

Key words : S-100 protein, blood-brain barrier, cerebral edema.

S-100 protein is a highly acidic protein first recognized as a soluble protein specific to the nervous system (11). Although some reports indicate its possible role as a membrane-bound protein (4, 6), nothing is yet known about its biological function.

Glial cells, the main location of S-100 protein in the brain (2, 7, 18), are believed to degenerate within a few minutes after the completion of cerebral arterial occlusion (5). This suggests that the S-100 protein content in a brain may change during the early stage of cerebral embolism. In the present report, the content of water-soluble S-100 protein in microembolized rat brain is investigated in relation to the development of cerebral edema.

MATERIALS AND METHODS

One hundred and eighty-three male and female Sprague-Dawley rats weighing 300 to 400 g were used in this study. All animals were kept in plastic cages with food and water *ad libitum*. The experiment was designed in two stages: one for S-100 protein measurement, the other for cerebral edema assessment after cerebral embolization.

Cerebral embolization was made by the injection of carbon microspheres ($35 \pm 5\ \mu$ in diameter) (8). The microspheres were suspended in rat serum (0.14 mg/0.07 ml) and were injected through the right common carotid artery after ligation of the right external carotid artery. A control experiment was performed, in which the same volume of rat serum was injected by the same method.

The animals for S-100 protein measurement were decapitated at 5 min, 2 h, 4 h, 8 h, 24 h and 48 h after the injection. The brains were removed immediately and were separated into the two cerebral hemispheres excluding the cerebellum. The whole procedure of removing the brain was accomplished within two minutes and the brain was stored

at −80℃ in all cases. For each survival period, 10 embolized and 10 serum-injected cerebral hemispheres were used for chemical and immunochemical analyses.

Quantitative microcomplement fixation assay (9) was employed for the measurement of water-soluble S-100 protein (20). Total soluble protein was estimated by the method of Lowry (10). The cerebral tissues were weighed and thawed immediately before the chemical and immunochemical analyses, then extensively homogenized in a glass hand-operated homogenizer in four volumes of cold barbital-NaCl buffer. The homogenates were centrifuged at 20,000 *g* for 30 min. The supernatants were processed for chemical and immunochemical analyses.

Monospecific antiserum against bovine S-100 protein was produced in New Zealand albino rabbits according to Plescia *et al.* (16). The antiserum, when examined by immunodiffusion (14), gave a single band with both purified bovine S-100 protein and the crude water-extract of rat brain.

The water content of brain was examined in 54 embolized and 9 serum-injected rats for the assessment of cerebral edema. Embolized animals were killed at 5 min, 1 h, 4 h, 8 h, 24 h and 48 h after microsphere injection. Nine control animals were killed immediately after serum injection. The water content was measured as wet-dry tissue weight (19).

RESULTS

Table 1 represents significant decrease in the S-100 protein content appearing at 4 h after microsphere injection. The maximum (71%) decrease in the content of S-100 protein was observed at 8 h after embolization, being a 67% decrease compared with that at 5 min after embolization. These are statistically significant at $P < 0.025$ value analysed by Student's *t* test. The content of S-100 protein had recovered by 24 h after embolization.

Table 2 shows the changes in the water content of rat brain after microsphere injection. The cerebral edema as judged by water content developed maximally at 8 h after an early transient increase in water content was observed at 5 min. The increase in water content except for the early transient increase correlated well with the decrease of S-100 protein. Moreover, the normalization of water content observed at 48 h corresponded well to the recovery of S-100 protein content.

TABLE 1. THE WATER-SOLUBLE S-100 PROTEIN IN RAT BRAIN[a] (μg/mg SOLUBLE PROTEIN)

Time after injection	Embolization	Serum injection
5 min	0.46 ± 0.14	0.43 ± 0.11
2 h	0.34 ± 0.15	0.37 ± 0.11
4 h	0.21 ± 0.11*	0.38 ± 0.14
8 h	0.15 ± 0.05*	0.51 ± 0.17
24 h	0.35 ± 0.15	0.41 ± 0.09
48 h	0.32 ± 0.10	0.45 ± 0.16

a Average of 10 animals ± standard deviation.

* Statistically significant at $P < 0.025$.

TABLE 2. THE WATER CONTENT IN RAT BRAIN[a] (%)

Time after inJecion	Water content[a] (%)
0[b]	77.6 ± 0.03
5 min	81.0 ± 1.80*
1 h	77.6 ± 0.06
4 h	78.6 ± 0.06*
8 h	80.4 ± 1.20*
24 h	78.7 ± 0.60*
48 h	77.9 ± 0.60

a Average of 9 animals ± standard deviation.

b Control group.

* Statistically significant at $P < 0.005$.

DISCUSSION

The present results agree with previous observations concerning the water content in embolized brain (8, 17). The early increase in water content at 5 min after microembolization had also been reported by Kogure and others (8).

The cerebral edema associated with a slight decrease in water-soluble S-100 protein content has also been observed in stab-wounded rat brains (15), suggesting that increase in water content and influx of serum protein into the cerebral parenchyma may account for the significant decrease in S-100 protein content. However, the 67% decrease in S-100 protein content observed in the present study can not be well explained by increased water content alone.

One explanation for the decrease of S-100 protein content is denaturation of S-100 protein faster than other total soluble proteins under the condition of cerebral ischemia. The authors observed that 50% of the antigenic activity of S-100 protein was lost after incubation of rat brains for 24 h at 37 ℃, while the amount of total soluble proteins was essentially unchanged even after the same treatment (13). The ischemic cerebral lesions produced by microembolization were estimated histopathologically as being less than 30% of the total hemisphere; therefore, rapid denaturation of S-100 protein can not be the main cause for its significant decrease after embolization. Moreover, the rapid denaturation does not explain the rapid recovery of S-100 protein content at 24 h after the embolization.

Another possibility is that water-soluble S-100 protein may bind to cytoplasmic membranes, resulting in its decrease during the development of cerebral edema. The reversible binding ability of S-100 protein to neuronal membranes (3) and its effect on the permeability of lipid membranes (1) strongly support this hypothesis. Thus, S-100 protein may bind to glial cytoplasmic membranes to regulate transmembrane transport during the development of cerebral edema.

The present results show that the content of water-soluble S-100 protein

correlates well with the development of cerebral edema in which the permeability of glial cytoplasmic membrane may be affected; however, further investigation regarding the role of membrane-bound S-100 protein is necessary to clarify the underlying mechanism.

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