Neuron-Specific Enolase Is a Marker of Cerebral Ischemia and Infarct Size in Rat Cerebrospinal Fluid

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Neuron-specific enolase concentrations were measured in samples of rat cerebrospinal fluid obtained repeatedly before and after occlusion of the middle cerebral artery. A method for reliable, repeated sampling of cisternal cerebrospinal fluid was developed for this purpose. Occlusion of the middle cerebral artery induced cerebral infarcts of slightly variable size with good correlation to raised neuron-specific enolase concentrations. Sham operation caused only superficial cortical damage at the site of surgery and was followed by an early, slight, and transient increase in neuron-specific enolase concentration. With our technique, the development of cerebral infarcts can be studied in individual rats under experimentally controlled conditions over an extended period of time. Analysis of neuron-specific enolase can be used in trials of drugs for mitigating the effect of ischemia. Information concerning the release of neuron-specific enolase from ischemic cerebral tissue to the cerebrospinal fluid is important because neuron-specific enolase in the cerebrospinal fluid can be determined in patients suffering from cerebrovascular insult. (Stroke 1988;19:1140-1144)

Neuron-specific enolase (NSE), the γ-subunit of enolase present mainly in neurons, and S-100 protein, a calcium-binding protein present mainly in glial cells, are regarded as nervous-system-specific proteins. 1,2 Both these proteins are cytoplasmic. Elevated NSE or S-100 concentrations in body fluids indicate the damage of cells expressing either of these two proteins. A correlation between NSE and S-100 concentrations in cerebrospinal fluid (CSF) and brain damage in patients with ischemic stroke and subarachnoid hemorrhage has indicated that these proteins can be valuable as markers of ischemic brain damage. 3-9 In these clinical studies the degree of brain damage was assessed by clinical status, computed tomography (CT) findings, or clinical outcome. The exact relation between the concentrations of NSE and S-100 protein in CSF and the structural changes in the brain cannot be established in detail in humans, and their temporal changes in CSF during the ischemic process remain incompletely understood.

Therefore, we used an experimental ischemic model in rats for the study of NSE concentrations in CSF in relation to the development and size of resulting infarcts. NSE was measured in CSF samples obtained from conscious, unrestrained rats subjected to focal ischemia by middle cerebral artery (MCA) occlusion. 10

Materials and Methods

We used 15 male Sprague-Dawley rats (310–370 g). They were allowed food and water ad libitum. During all surgical procedures the rats were anesthetized by intraperitoneal injection of 1.3–1.5 ml of a mixture containing 4.25% chloral hydrate and 0.97% pentobarbital and were breathing spontaneously. After MCA occlusion, 20 ml Ringer’s solution was injected subcutaneously.

Cannulation of the cisterna magna and sampling of the CSF were performed as described by Sarna et al., 11 with certain modifications. The catheter was introduced into the cisterna magna by one of two routes, subdural or extradural. For subdural introduction in eight rats (Figure 1), a burrhole 2 mm in diameter was drilled in the midline immediately anterior to the external occipital crest. The dura was pierced, and the...
catheter was directed caudally in the direction of the cisterna magna. For extradural introduction in seven rats (Figure 2), the burrhole was made slightly more caudally in the midline, directly over the external occipital crest. The catheter was inserted extradurally and pushed caudally along the inner surface of the occipital bone. The dura was thus perforated 1–2 mm above the foramen magnum, and the catheter was inserted directly into the cisterna magna.

We analyzed 121 CSF samples; 50–75 μl CSF was gently aspirated at intervals after insertion of the catheter via a connected catheter. The rats could move freely in their cages during this procedure. Care was taken to flush slowly any CSF remaining in the catheter back into the cisternal space before the catheter was sealed. Additional CSF samples were obtained during the following 1–2 days to confirm that the system was patent; if CSF was easily obtained the rats were assigned to experimental treatment: MCA occlusion, sham operation, or CSF sampling alone. CSF was then sampled each day until the rats were killed.

CSF samples were centrifuged for 5–10 minutes at 5,500 rpm, and 50 μl of the supernatant fluid was frozen at −70°C. NSE content was analyzed by radioimmunoassay (RIA) (Pharmacia AB, Uppsala, Sweden).12 In this RIA, human NSE is used as a standard. All NSE analyses were performed blind-coded. The data were analyzed on a personal computer using commercial software.

The MCA was occluded using a technique described by Tamura et al.10 Care was taken to coagulate the MCA, starting proximal to the olfactory tract and lenticulostriate artery and proceeding to the inferior cerebral vein.13 The neurologic status of the rats was assessed each day after the occlusion.13 A sham operation was performed in an identical manner except that the brain surface was slightly coagulated without touching the MCA.

The rats were anesthetized and perfused through the heart with 200 ml of 4% buffered formaldehyde. The brain was left in situ and immersed in fixative overnight. Coronal sections of the brain were cut, embedded in paraffin, and stained with hematoxylin and eosin and van Gieson’s stains. All histologic sections were examined without prior knowledge of the experimental treatment.

**Results**

All rats lost some weight after catheter implantation but showed no other signs of distress. Eight

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**FIGURE 1. Subdural catheter implantation in rats. Neuron-specific enolase (NSE) concentration curves show high but rapidly decreasing concentrations in cerebrospinal fluid shortly after catheter implantation.**

**FIGURE 2. Extradural catheter implantation in rats. All but one cerebrospinal fluid sample contained <4.4 ng neuron-specific enolase (NSE)/ml (normal value).**
catheters became occluded within 4 days, and seven remained patent for >7 days. Normal NSE concentration in CSF obtained by a direct puncture of the cisterna magna is <4.4 ng/ml.

To evaluate the two catheter implantation techniques NSE was measured in 70 consecutive CSF samples from 15 rats (including preoperative values from five rats later subjected to craniotomy). NSE was measured in 41 consecutive samples obtained up to 9 days after subdural introduction of the catheter (Figure 1). The NSE concentration was high in most samples obtained within 24 hours (mean, 42.7 ng/ml). Subsequently the NSE concentration decreased and became approximately normal within 48 hours. Necropsy showed that the catheters had passed through and damaged the posterior part of the cerebellum before reaching the cisterna magna. NSE

**FIGURE 3.** Neuron-specific enolase (NSE) concentration curves in four middle cerebral artery-occluded (Rats 2–5) and one sham-operated rat (Rat 1).

**FIGURE 4.** Light microscopic appearance of infarct in caudoputamen. Note extensive necrosis and dense infiltration especially by macrophages.
concentrations in 29 consecutive samples obtained up to 11 days after extradural introduction of the catheter were normal in all but one rat (8.4 ng/ml) (Figure 2). Postmortem examination showed that no catheter had destroyed any part of the cerebellum.

Four rats were subjected to MCA occlusion and one to sham operation. CSF was sampled from each rat over an average of 7 days after surgery. Neurologic examination of all four rats subjected to MCA occlusion disclosed evidence of a focal ischemic lesion, with diminished resistance to lateral push and forelimb flexion. The sham-operated rat was neurologically normal.

Following MCA occlusion, NSE concentrations rose to a maximum after 24–72 hours. Concentrations then decreased and returned to roughly normal after 6 days (Figure 3). NSE concentrations in the sham-operated rat were lower, reaching a maximum within 24 hours and returning to normal after 4 days.

Areas under the NSE concentration curves were integrated (from the day of surgery to Day 5) and were found to be 8.7 area units in the sham-operated rat (Rat 1) and 21.3, 22.0, 26.9, and 33.2 area units in Rats 2–5, respectively, in the MCA-occluded rats.

The brains of all four MCA-occluded rats showed areas of cerebral infarction characterized by ischemic necrosis, degenerative changes in neurons and glial cells, and macrophage infiltration (Figure 4). The infarcts were located in the ipsilateral frontoparietal cortex and in the caudoputamen, particularly in the lateral part (Figure 5). All layers of the cerebral cortex were affected, all the way to the surface. The thalamus, hippocampus, and white matter showed only slight or no changes. The sham-operated rat showed only slight local changes in the exposed cortex but no infarction.

The size of each infarct was estimated from camera lucida drawings of a coronal section of the brain at the level of the bregma. Area of the infarct was expressed as percent of the area of the entire section (relative size). A significant correlation ($r = 0.97, p<0.01$) emerged between the relative size of the infarct and the NSE concentration in individual rats (Figure 6).

Discussion

Our study shows that consecutive analysis of NSE released to the CSF from damaged cerebral tissue reflects the development and size of infarcts in the MCA occlusion model in rats. Temporal variations in NSE concentration indicate that the ischemic process is dynamic and that assessment of intrindividual variations is possible. The time course of all NSE concentration curves was apparently similar, but the maximum occurred later for larger than for smaller infarcts (48–72 hours vs. 24 hours). Our findings indicate that neuronal damage in focal ischemia may continue to develop for several days after an insult (see References 14 and 15). Our techniques allow the study of the temporal course of delayed neuronal death. Furthermore, analysis of NSE concentrations in CSF can be used to study temporal effects on the development of the infarct and its final
size under various experimental conditions and to evaluate therapeutic trials on living animals.

MCA occlusion induced infarcts of slightly varying size. Both the cortex and the caudoputamen were affected in all rats. Histologic examination disclosed ischemic brain damage closely resembling human infarcts, consisting of a well-delineated area of necrosis, nerve and glial cell changes, and macrophage infiltration. The size of the infarcts was estimated from sections cut at the level of the bregma because infarcts proved to have their greatest extension at this level. Close correlation was found between the area under the NSE concentration curve and the relative area of the infarct at the level of the bregma, indicating a strong correlation between NSE concentrations in CSF and infarct size.

Much research has been done to find clinically useful CSF markers of ischemic brain damage, such as NSE and S-100 protein, that could be used to assess structural brain damage during the acute phase of a disease. Although many modern imaging techniques such as CT, positron emission tomography, and magnetic resonance imaging yield important information concerning the status of the brain in ischemic situations, they cannot distinguish between irreversibly damaged brain tissue (infarcts) and reversible changes in viable tissue (edema). In a clinical assessment during the acute phase of an ischemic insult it is also difficult to distinguish between reversible and irreversible cerebral changes. For example, to differentiate between transient ischemic attacks, reversible ischemic neurologic deficit, and cerebral infarction, clinical observation for several days is needed. The analysis of nervous-system-specific, cell-damage-related proteins such as NSE may contribute to the more detailed estimation of the actual structural brain damage in various clinical situations.

Our study provides experimental evidence that the release of NSE into the CSF reflects in quantitative terms the ischemic process and the final size of structural damage in the brain. The area under the NSE concentration curve seems to be a useful measure of the extent of ischemic damage. In a recent clinical study we found a relation between CSF S-100 protein concentration curves and the clinical outcome in patients with subarachnoid hemorrhage; this indicates that the area under the concentration curve is a measure of brain damage. The time of CSF sampling in relation to ictus is of crucial importance when evaluating a series of CSF samples.

NSE cleared rapidly from the CSF in subdurally inserted catheters causing mechanical cerebellar damage, which implies that the kinetics of NSE concentrations in CSF differs in traumatic and ischemic lesions. Our findings also emphasize the importance of nontraumatic cisternal catheter implantation in rats and suggest that an extradural suboccipital approach is to be preferred because this was not associated with an increase in NSE concentration. NSE analysis can thus also be used to assess the skill of catheter implantation in an individual animal.

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References


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