Uptake of plasma proteins into damaged neurons
An experimental study on cryogenic lesions in rats
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Summary. Preliminary observations on human autopsy material have indicated that damaged neurons may take up plasma proteins early after the injury. These observations prompted an experimental study under controlled conditions. Focal brain lesions were produced in rats by extracranial application of dry ice for 90 s. This caused an immediate disruption of the blood-brain barrier with leakage of plasma components into the tissue and sharply circumscribed areas of necrosis of the underlying cortex. Five minutes after the lesion, uptake of albumin, fibrinogen and fibronectin into damaged neurons was demonstrated by immunostains. These proteins were retained in the injured neurons until they were phagocytized 2–4 days later. In addition, normal neurons whose axons or axon collaterals passed through or terminated in the lesion were labeled. This labeling was generally weaker than in damaged neurons and no labeling of neuronal nuclei was observed in these cells in contrast to those of damaged cells. Apart from nerve cells labeled through retrograde axonal transport, no staining of normal neurons was observed. Intravenous injections of Evans blue, which binds to plasma proteins, confirmed that albumin was taken up into damaged neurons almost immediately after the injury and showed that this uptake continued for at least 20 h. It is concluded that uptake of plasma proteins into damaged neurons may serve as early (and late) markers of neuronal injury.

Key words: Blood-brain barrier – Necrotic neurons – Immunohistochemical neuronal markers – Axonal transport

Cryogenic brain lesions have been used in numerous investigations to study the spread of vasogenic brain edema (e.g. [1, 4, 13, 14, 27, 28]). Uptake of macromolecules into damaged neurons has also been described in this model [13, 27, 28], as well as in other conditions with damaged blood-brain barrier (BBB) [5, 10, 19, 23, 25, 29], but little attention has so far been paid to this phenomenon.

We have recently observed uptake of plasma proteins into damaged neurons in human brain contusions and following anoxic lesions, and our findings suggest that this may be used as an early marker for neuronal injury. Because of uncertainties concerning the effect of autolysis and concerning the exact intervals from the onset of the lesion to death in human cases, we wanted to study this phenomenon under controlled experimental conditions. Since cryogenic lesions cause immediate leakage of proteins, we decided to use this model to elucidate the following questions:
1. How soon after the injury does uptake of plasma proteins into damaged neurons take place?
2. Do damaged neurons retain the plasma proteins until they are removed by phagocytosis?
3. Will damaged neurons be saturated with proteins shortly after the injury or does the uptake continue over some time?
4. Do normal neurons or other normal cells take up proteins when they are surrounded by protein-rich edema fluid?

Material and methods
Thirty 200- to 250-g female Wistar rats (Møllegaard, Copenhagen, Denmark) were used in the study. All animals were anesthetized with a combination of fentanyl (0.5 mg/ml) and fluanison (2.5 mg/ml; Hypnorm, Janssen Beers, Belgium) and midazolam (1.25 mg/ml; Dormicum, Roche, Basel, Switzerland; injected subcutaneously (6.0 ml/kg body wt.). Cryogenic lesions were produced in 27 animals while three normal animals served as controls.

Surgical and histological procedures
The cryogenic lesions were produced after exposure of the calvarium in the right parietal region. A tip of dry ice (−70°C) was applied against the intact bone for 90 s. This resulted in a cortical
lesion with a diameter of approximately 4.5 mm. The animals were killed at intervals from 5 min to 8 days (d) after the lesion (5 min, 30 min, 1 h, 6 h, 12 h, 24 h, 2 d, 4 d and 8 d). The brains were fixed by perfusion through the heart with 4% phosphate-buffered formaldehyde, and the fixed brains were cut coronally through the middle of the lesion. In 20 animals, blocks from the lesions were embedded in paraffin and cut at 4 μm. Blocks from the control animals were treated in the same way. Immunostaining was then performed as described below. Hematoxylin and cosin (H&E) was used as a routine stain.

Evans blue was injected into the tail vein in seven animals (2%, 3 ml/kg body wt.) Four received the injection 30 min before the cold injury. Two were killed after 5 min, one after 1 h and one after 1 day. One animal received the injections 3 h and two 20 h after the injury. They were killed 24 h after the injury. The fixed brains from these animals were frozen in liquid nitrogen. Ten-micrometer-thick cryostat sections were then cut and examined in the fluorescence microscope.

Immunostaining was performed with antisera against human albumin, fibrinogen and fibronectin (Dakopatts A/S, Glostrup, Denmark) and with antiserum against rat albumin (Nordic Immunological Laboratories, Tilburg, The Netherlands). In a small number of animals, immunostaining was also performed with antisera against S-100 protein and GFAP (Dakopatts A/S). The antigen-antibody reaction was visualized with the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method using fast red as the chromogen.

Results

General histology

The general histopathological features within and outside the lesions were studied in H&E-stained sections.

Fig. 1a-f. H&E-stained sections from cold lesions. a One hour after injury. b Higher magnification from the same section showing the vaculated zone between lesion (left) and the normal cortex (upper right). c Shrunken neurons with condensed nuclei 5 min after the injury. d Normal cortex for comparison. e Pale and slightly swollen neurons 1 h after the injury (arrows). f Dark shrunken neurons at the edge of the lesion 30 min after the injury. a × 13; b × 80; c-f × 500