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Cellular reactions to implantation of a microdialysis tube in the rat hippocampus*

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Summary. Microdialysis tubes, used for measurements of extracellular neurotransmitter concentrations, were implanted in rat dorsal hippocampus to study the adjacent tissue reaction. The brain was examined 1–60 days after the implantation. Within the first 2 days, normal neuropil and only occasional hemorrhage surrounded the microdialysis tube. Three days following the implantation astrocytes close to the dialysis tube, hypertrophied. Hypertrophic astrocyte processes invaded the spongy fiber wall. There was no increase in the number of astrocytes. Fourteen days after the fiber insertion layers of reticulin-positive fibers separated astrocytes and the remaining neuropil from the fiber wall. Late tissue changes (1 and 2 months) consisted of collagen deposits and occasional granuloma formation. These results can be used to predict the optimal time for commencing microdialysis after the fiber implantation.

Key words: Microdialysis – Hippocampus – Tissue reaction

In vivo brain microdialysis provides a means by which extracellular concentrations of small molecular weight substances can be measured continuously over several hours [14, 19]. A thin (300 μm) semipermeable hollow fiber is stereotaxically positioned through a brain region of interest and perfused at a constant flow rate. The perfusate is collected for HPLC analyis. The technique makes it possible, biochemically, to distinguish extracellular from intracellular compartments. Microdialysis has provided results such as increased concentrations of glutamate, aspartate, GABA and taurine during kainic acid perfusion [10], during global cerebral ischemia [1], hypoglycemia [15] and electroconvulsive shock [9]. Solute diffusion from the extracellular space to the fiber depends on fiber membrane permeability, fiber perfusate composition, fiber flow rate and presumably also the chemical composition of the surrounding tissue. During steady state conditions extracellular amino acid concentrations in collected dialysate reflect levels in brain compartments adjacent to the dialysis tube. However, studies involving chronic microdialysis may face the problem of changed tissue composition adjacent to the fiber (gliosis, oedema, necrosis and hemorrhages, etc.) The likelihood of changed diffusion characteristics due to the interposition of reactive tissue, should be given serious consideration when planning chronic experiments. In addition other problems might arise if a certain substance secreted by neuronal tissue is either produced or metabolized by surrounding glia cells or histiocytes. This study defines histologically the time course of tissue changes surrounding a microdialysis fiber implanted in the rat dorsal hippocampus. These morphological results define, together with autoradiography of glucose metabolism and blood flow the optimal time for commencing microdialysis after the implant procedure.

Materials and methods

Implantation procedure

Thirty-eight male Wistar rats (350–400 g) were anestetized with Pentobarbital (50 mg/kg i.p.) and placed in a stereotaxic instrument (David Kopf, small animal instrument, model 900 and rat adapter 920). A mid-line incision was made in the scalp and both temporal muscles were carefully removed. With a spherical dental drill 2-mm burr holes were made bilaterally (3.0 mm below and 3.0 mm behind Bregma) in the temporal bone. Continuous cooling with saline was used. Supported by a 150-μm-thin inner steel wire, a dialysis fiber (Diaflo Hollow Fiber

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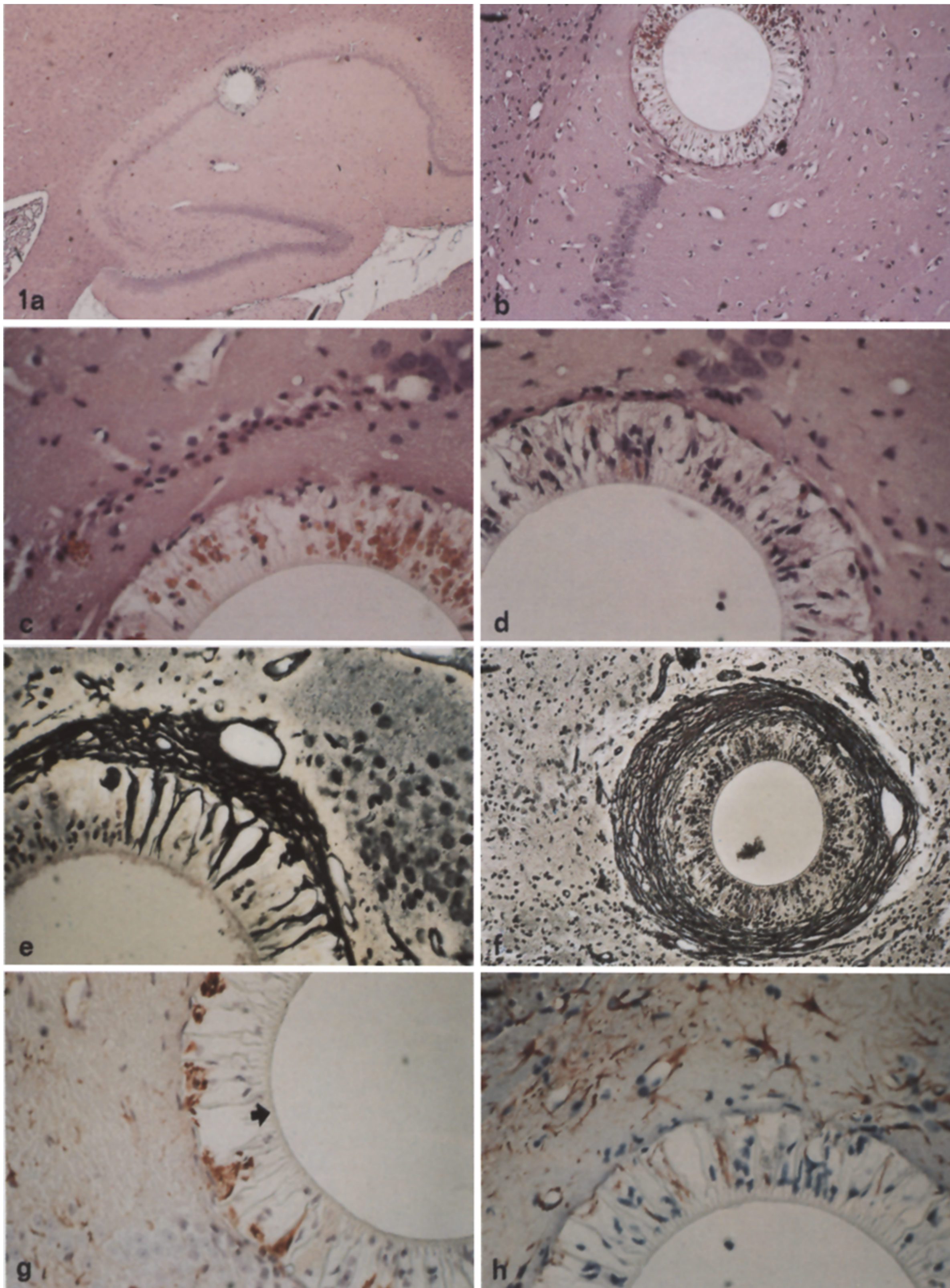

Fig. 1. a The microdialysis tube positioned in the dorsal hippocampus, 24 h after the implantation, H&E, ×27. **b** The same figure in a higher magnitude shows erythrocytes and polymorphonuclear leucocytes in the spongy fiber wall, ×96. **c** Eosinophilic neurons lying about 50 μm from the microdialysis tube in hippocampus. The spongy fiber wall is infiltrated with erythrocytes, 24 h following implantation, H&E, ×306. **d** Four days after the implantation of the fiber, macrophages, lymphocytes and multinucleated giant cells invade the fiber wall, H&E, ×306. **e, f** Reticulin-positive fibers surrounds the microdialysis tube in two rats killed 2 weeks following implantation, **e** ×306, **f** ×105. **g, h** Increased glial fibrillary acidic protein 6 days and 14 days following implantation. Astrocyte processes are seen in the sponge. The *arrow* points at the dialysis membrane on the interior which provides the barrier of selective permeability, ×306

H1 × 50, molecular weight cut-off, 50,000; Amicon) [18] was inserted through the burr holes aided by the micromanipulator of the stereotaxic instrument. The skin wound was closed with silk sutures (3.0). The microdialysis tube was then flushed gently with Krebs-Ringer bicarbonate.

Preparation of tissues

The rats were deeply anestetized with 3% halothane. The chest was widely opened. The right and left ventricle were quickly incised and a cannula was thrust into the ascending aorta. Within 20 – 30 s after the chest had been opened 500 ml of Lillis phosphate-buffered formalin, delivered via a roller pump, was run into the vascular tree. The skull was opened 60 min after fixation, and the brain was removed and further fixed for 24 h in formalin. After dehydration and paraffin-embedding 5-μm sagittal sections were cut and stained with hematoxylin-eosin, cresyl-violet, a.m. Klüver-Barrera, a.m van Gieson, for reticulin and glial fibrillary acidic protein (GFAP) [6].

Results

Clinical observations

All animals survived the fiber implantation. No signs of seizure activity, paresis or behavioral alterations were observed postoperatively.

Tissue changes 1 and 2 days after implantation

Ten animals were studied. In the fiber wall, scattered accumulations of extravasated erythrocytes and polymorphonuclear leucocytes were seen (Fig. 1 b). The distribution of hemorrhage, polymorphonuclear leucocytes and edema varied along the microdialysis tube. Some parts demonstrated heavy infiltration, other parts none. In brain tissue adjacent to the fiber (a 50-μm border zone), areas of edema (vacuolization) and sometimes minor hemorrhages were characteristic. Eosinophilic neurons were occasionally present within 100 – 150 μm from the implant (Fig. 1 c). Few neurons (a 100-μm perimeter from the implant) showed signs of pyknosis or karyorrhexis.

Tissue changes 3 – 14 days following implantation

In the 20 animals studied, hemorrhage and edema progressively diminished. Phagocytotic cells (macrophages and multinucleated cells) invaded the spongy wall of the microdialysis tube (Fig. 1 d). The cytoplasm of macrophages indicated ingestion of debris (sometimes hemosiderin). In this phase the fiber wall in its full extension was occupied by phagocytes and the reticulin stain showed several layers of reticulin fibers (Fig. 1 e, f).

Viable neurons were seen adjacent to the tube (in case of formation of granulation tissue within 200 – 300 μm from the tube). Microvacuolization also characterized the tissue in vicinity of the tube. The first signs of increased GFAP appeared 3 days following implantation. No mitotic activity was seen. The astrocytes had hypertrophic processes extending towards and within the fiber wall (Fig. 1 g, h). No correlation between the extension of astrocytic reaction and time of implantation was found. However, areas of hemorrhage and hypertrophic astrocytes seemed to coincide. Areas dominated by phagocytotic activity coincided with large areas of hypertrophied astrocytes.

Tissue changes 30 – 60 days following implantation

One to two months following the implantation chronic granulomatous changes were found in eight animals. The changes were typical of granulomas seen in vicinity of foreign bodies and surrounded the microdialysis tube in a 2-mm wide zone. The majority of cells consisted of macrophages and giant cells. The outer perimeter was dominated by lymphocytes and a zone of reactive astrocytes. Collagen surrounded the fiber wall and extended into the sponge.

Discussion

The present study demonstrated minimal tissue changes within 1 to 2 days following the implantation. After 3 days astrocyte hypertrophy was present but did not progress; there was no numeric increase of these cells. Connective tissue replaced astrocytes after 2 weeks and was still present 60 days later.

In a now classical study Del Rio Hortega and Penfield [5] studied tissue changes in the vicinity of experimental cortical lesions and determined the extent of gliosis and scar tissue formation in necrotic areas. Astrocytes proliferated on the 4th day followed by extensive scarring. In contrast, much less gliosis was observed if damaged tissue was completely removed from the site of the lesion [12, 13]. The same principles apply with respect to tissue reactivity in the vicinity of brain implants, i.e., atraumatic surgical procedures produce minimal gliosis and scarring [16]. In contrast the introduction of a rather large needle (2.4 mm, outside diameter) into the rat brain causes pronounced gliosis [3].

The inhomogenous distribution of hypertrophic astrocyte processes found in this study probably correlates with the unavoidable mechanical trauma during surgery causing extravasation of erythrocytes and edema in the first 2 days. The dialysis membrane and surrounding neuropil are, in the majority of implantations, completely coherent and only sep-

arated by the sponge which makes up the fiber wall. As such the method is superior to previous techniques (i.e., push-pull cannula [7], the cortical cup [11]).

Astrocytic hypertrophy or actual proliferation depend not only on the degree of trauma during the insertion of a brain implant, but perhaps also on the type of material used. The insertion of a microdialysis tube in this study made of acrylic copolymer initiates astrocyte hypertrophy but no numeric increase in these cells (gliosis). Similar results are found when araldite-coated implants [16] or stainless steel electrodes [4] are used. In contrast, cuprophan, which has been used in microdialysis, apparently induces both pronounced gliosis and also the formation of endothelial cell layers [8]. It is possible that these tissue reactions are caused by the material itself or that the implantation procedure is surgically more traumatic to the tissue. Compared to other methods microdialysis causes only discrete tissue changes. No doubt these may be minimized even further if the utmost care is taken during surgery.

An important point, however, is whether the method changes the normal physiochemical properties of brain tissue. Recent studies have shown that glucose metabolism and local cerebral blood flow is disturbed 2 hours following the implant. In a 1-mm wide tissue cylinder surrounding the fiber one finds areas of increased glucose metabolism and decreased blood flow. This situation is normalized after 24 h [2]. Blood-brain barrier integrity is unaffected, at least acutely [1, 17], and one can assume, therefore, that there is no leakage of plasma components from the systemic circulation. Depending on the type of study, the use of microdialysis for chronic experimentation (after 2–3 days) may become unreliable. This was illustrated by Korf and Venema who found a steady increase in the resting release of several amino acids over a period of 9 days [9]. The same authors also found different responses to high potassium infusion and electroconvulsive shock. It is likely that both hypertrophied astrocytes and inflammatory cells can modulate biochemical reactions originating from a particular insult. With the invasion of inflammatory cells into the fiber wall as well as the coating with connective tissue, diffusion characteristics of the microdialysis tube can be changed. The administration of drugs should still be possible, since these in most cases will diffuse beyond the rim of changed tissue. As yet no concrete information is available on these issues.

The optimal time for commencing microdialysis after the implant appears to be between 24–48 h. At this time tissue changes are minimal and the cerebral glucose metabolism and blood flow are normal. The reliability of the microdialysis technique for chronic studies is questionable, since no information is available on the impact of the more pronounced tissue changes.

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