Short report

Postmortem survival characteristics of rat glial cells in culture

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SUMMARY Cell-type-specific markers and indirect immunofluorescence were used to determine the longest time interval between death of the animal and removal of tissues that permitted growth in vitro of rat central glial cells. Galactocerebroside⁺ oligodendrocytes could not be cultured after more than 1 hour had elapsed, whereas small numbers of glial fibrillary acidic protein (GFAP)⁺ astrocytes and GFAP⁻ fibroblastic cells could still be cultured for as long as 6-7 hours after death. These observations may reflect a greater susceptibility of oligodendrocytes to anoxia compared with astrocytes and fibroblasts.

In tissue culture experiments using tissues of nervous system origin it is common practice to kill animals individually rather than in batches prior to culture. This is generally assumed to be sensible because of the likely effects of anoxia on tissues with consequent reduction of the quality of the cultures which may result if large numbers of cadavers are left for prolonged periods on the laboratory bench before dissection. Moreover, it seems logical to assume that different cell types would vary in the extent to which they can withstand anoxia and, therefore, that the cellular composition of cultures would vary following different periods after death. To test these possibilities cell-type-specific markers and indirect immunofluorescence were used in this study to determine how long different neonatal rat brain glial cell types survive following death, survival being defined by the ability of these various cell types to grow in tissue culture.

Methods

The techniques used in the study have been previously described in detail.¹⁻³ All experiments were performed on 6 day old Wistar rats. The animals were killed by decapitation

and the heads were left at room temperature for periods ranging from 0-15 hours (0, 1, 2, 3, 4, 5, 6, 7, 8 and 15 hours prior to dissection). These experiments were performed on three separate occasions and the brains of two animals were used for each time point. The corpus callosum was dissected aseptically and put into tissue culture as previously described.^{1,3} In brief, corpus callosum tissue from each pair of animals was placed in MEM-HEPES, chopped into small pieces with scissors and digested for one and a half hours at 37°C in 0.125% trypsin and 0.02% collagenase. The cells were then dissociated by repeated trituration through a Pasteur pipette and the resulting cell suspension was plated on 13 mm glass coverslips (20,000 cells per coverslip) in Dulbecco's Modified Eagle's Medium containing 10% foetal calf serum in Linbro Multiwell plates. The cells were grown in a humidified atmosphere of 90% air:10% CO₂ at 37°C and the medium changed every 2 days. Cells were studied after 5-7 days in culture.

Cell identification in these cultures was carried out using indirect immunofluorescence and cell-type-specific markers as previously described.^{1,2} Astrocytes were identified by using a rabbit antiserum against glial fibrillary acidic protein (GFAP)^{1,4} (rabbit anti-GFAP, diluted 1:100), oligodendrocytes using a rabbit antiserum directed against galactocerebroside² (rabbit anti-GC, diluted 1:40), and fibroblasts by mouse monoclonal antibodies to Thy 1.1¹ (mouse anti-Thy-1.1). Antibody binding was visualised with goat anti-rabbit IgG conjugated to rhodamine (G anti-Rlg-Rd, Nordic) or goat anti-mouse IgG conjugated to fluorescein (G anti-Mlg-FI, Nordic). Immunofluorescence assays were performed as previously described.^{1,3} Cells growing on glass coverslips were incubated with the primary antibody for 25 minutes at room temperature, washed and

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Table In vitro survival of neonatal rat central glial cells following death of the animal

Hours following death	GC ⁺ oligodendrocytes*	GFAP ⁺ astrocytes	GFAP ⁻ fibroblastic cells
0	+	+	+
1	+	+	+
2	—	±	±
3	—	±	±
4	—	±	±
5	—	±	±
6	—	±	±
7	—	—	±
8	—	—	—
15	—	—	—

*Cell growth was assessed on a simple arbitrary scale:
+ denotes good growth of cells with cultures containing approximately 5% GC⁺ oligodendrocytes, 40% GFAP⁺ astrocytes and 50% GFAP⁻ fibroblastic cells. At 0–1 hours the total number of cells per coverslip was more than 5,000 cells after 5 days in culture.
± denotes sparse cell growth. At and after 2 hours the total number of cells per coverslip was usually less than 200 after 5 days in culture.
— denotes absence of any cell growth.

then incubated with either G anti-Rlg-Rd or G anti-Mlg-FI for 25 minutes at room temperature. After washing the cells were fixed in acid-alcohol (95% ethanol: 5% glacial acetic acid) at -20°C for 10 minutes, washed and mounted in glycerol on glass slides. The cells were then examined under a Zeiss Universal fluorescence microscope equipped with epillumination and phase contrast optics. At least 200 cells per coverslip were counted. For staining with GFAP the cells were prefixed in acid-alcohol prior to staining.

Results

The results of these experiments are summarised semi-quantitatively in the table. In cultures obtained from tissues at 0 hours and 1 hour after death, 5% of the cells were GC⁺ (positive) oligodendrocytes, 40% were GFAP⁺ astrocytes and 50% were GFAP⁻ (negative) Thy.1⁺ fibroblasts, consistent with previous detailed studies.^{1,3} There was no difference in the quality of the cultures or the proportions of the various cell types at 0 and 1 hours, and the total number of cells per coverslip was 5,000 or more at both these time periods. After the rat heads had been left for 2 hours at room temperature it was no longer possible to culture GC⁺ oligodendrocytes and only small numbers of GFAP⁺ cells or GFAP⁻ fibroblastic cells were detected, the total number of cells per coverslip seldom exceeding 200. This pattern of sparse cell survival continued to be observed until 7 hours after death when it was no longer possible to grow GFAP⁺ cells although a few GFAP⁻ fibroblastic cells could still be cultured. By 8 hours following death it was not possible to grow any neural cell types in culture.

Discussion

These experiments show a differential survival pattern

of rat brain glial cells in culture following death of the animal. No GC⁺ oligodendrocytes could be grown after more than 1 hour had elapsed after death, whereas both GFAP⁺ astrocytes and GFAP⁻ fibroblasts could be grown in culture after as long as 6 or 7 hours after death. All of the cell cultures obtained after 2 hours contained far fewer cells compared with the cultures prepared at early time intervals. These differences in survival times may well reflect different sensitivities of these cells to anoxia; oligodendrocytes appeared to withstand anoxia much less well than did astrocytes and fibroblasts. An additional factor in the poor survival of oligodendrocytes after 1 hour is that the viability of these cells in culture may be critically dependent on the numbers of other cell types present such as astrocytes and fibroblasts, which were also considerably reduced after this time.

These observations may be of particular relevance to human cell cultures where conflicting results have been obtained. In previous studies^{5–7} success in preparing neural cell types from aborted human fetuses varied according to the source of the material. In the case of vacuum-extracted fetuses, which were almost always cultured within 2 hours of death, the success rate was very high (more than 90%). On the other hand, prostaglandin-induced fetal material, which may well have died several hours before delivery, could only be grown in culture in about 40% of cases. In the latter case it is possible that the relatively few successful cultures had been prepared from fetuses which had died within 2 hours of delivery. Moreover, even in "unsuccessful" human cultures, a very small number of GFAP⁺ astrocytes and GFAP⁻ fibroblastic cells could be grown in culture even when GC⁺ oligodendrocytes could not be grown,⁶ providing further evidence of the greater susceptibility of oligodendrocytes to anoxia.

Lewis and McCoy⁸ used histological methods to determine the periods of survival of several different types of cell from a variety of rat tissues after death. The criterion for survival was the presence of intracellular granules and vacuoles which had an affinity for neutral red; when the tissues died the granules and vacuoles ceased to be labelled with this dye which then diffused throughout the cell. Using this method it was found that although some tissues such as kidney epithelium and smooth muscle survived for up to ten days after the death of the animal, nerve cells from the cerebral cortex and cerebellum only survived for 1 hour. Alvarez⁹ reported that the survival rates after death of a variety of tissues could be extended by refrigeration at 0°C prior to culture. Murray and Stout¹⁰ confirmed this study for neural tissues by showing that after storing sympathetic ganglia at 4°C many hours after the latter had been removed from patients at operation it was still possible to culture

human peripheral neurons. In addition it has been claimed that by keeping tissues refrigerated at 6°C¹¹ it was possible to grow human fetal neural cells in culture up to 9 days after the nervous tissue had been removed from the fetuses. However, the various neural cell types in these experiments were not positively identified with specific antisera. In limited experiments using human fetal tissues it was possible to obtain reasonably good cell cultures of astrocytes, oligodendrocytes, fibroblasts, peripheral neurons and Schwann cells from vacuum-extracted dorsal root ganglia and spinal cord tissues after they had been stored overnight at 4°C in buffered medium (MEM-HEPES).⁶

The main practical implication of the experiments described here with neonatal corpus callosum cultures is that when large numbers of animals are being killed for the preparation of nervous system tissue cultures, they should be killed individually prior to dissection and not killed in batches and left at room temperature for prolonged periods of time. If there are to be delays in dissection once the animals have been killed then it seems sensible to store the cadavers at 4°C. Moreover, tissue culture work is now being widely used in both experimental and clinical research. For example, biopsy material is being studied from human brain tumours and benign brain lesions.¹² The results reported above may have relevance to these studies in that the pattern of cell survival in cultures derived from these sources may be directly affected by the time interval between obtaining tissues and cooling or establishment of cultures.

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