Cytological Postmortem Changes in Highly Specialized Cells Subjected to Different Causes of Necrosis

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Emphasis in cytological research has traditionally been on representing and understanding cells and their processes in the living state; changes occurring in the cell after death were considered unphysiological and uninteresting. Yet, with the attention programmed cell deaths have received lately (Duke *et al.* 1996, Fraser *et al.* 1996, Korsmeyer 1995, Martikainen 1996), there comes a need to know more about accidental cell death as well.

It could be argued that if programmed cell deaths, until very recently clumped together under the term “apoptotic”, do not constitute a homogeneous category, but may be classified on the basis of highly diagnostic ultrastructural images as a) apoptosis, b) atrophy, and c) differentiation (Bowen *et al.* 1996), then accidental cell deaths may also fall into a variety of categories. Unfortunately, the prevailing view is still that accidental cell deaths collectively conform to a single form of ‘necrosis’. The latter is thought to pass along the following regular chain of events: calcium overload with subsequent cell oedema, dilation of endoplasmic reticulum, polysome disintegration, and mitochondrial swelling (Bowen *et al.* 1996).

When we noticed that in rabbit photoreceptors postmortem changes affecting visual and glial cells were most dramatic within the first two hours following death (Pow and Crook 1994), but the time-course for dying visual cells in the fly eye was different (Meyer-Rochow *et al.* 1996), we decided to take a closer look at whether necrotic photoreceptor cell deaths really were uniform in their ultrastructural manifestations, given different causes for their demise.

The choice of the experimental cell type fell on the fly photoreceptor cell, firstly because a great deal is known about the ultrastructural organization of normal, healthy fly eyes and secondly because for studies on photoreceptor dystrophies and programmed cell deaths the eye of the fruitfly *Drosophila* had previously been used as a model (Stark *et al.* 1995). Furthermore, since studies on visual parameters are often based on results obtained from eyes of isolated fly-heads, even many hours after decapitation, it seemed useful to know to what extent the ultrastructures of such eyes differed from those that were fixed immediately.

Material and methods

Four groups of 10 apparently healthy and similarly-aged individuals of the fly *Calliphora erythrocephala* (irrespective of sex) during the day in light were subjected to: 1. decapitation, 2. ethyl-ether, 3. gaseous CO₂, and 4. KCN-vapour. Following these ‘regimens’, 15 and 30 min as well as 3, 6, and 24 hr later after illuminated storage in covered Petri dishes (to keep humidity constant in the case of the isolated heads), fly eyes were prepared for electron microscopic examinations in the usual way, i.e. prefixation with phosphate-buffered (pH 7.4) glutaraldehyde-paraformaldehyde, fixation in 1%-buffered OsO₄, dehydration, and embedding in resin.

In order to compare the ultrastructures of the photoreceptor cells from the four groups,

care was taken that only sections through identical areas of the eyes were chosen. Furthermore, since photoreceptive membranes (as the major sites of the photopigment molecules) and mitochondria (as the major suppliers of energy) hold key-positions in the functional viability of the photoreceptor cell, these structures in particular were made the subjects of the comparative analysis of postmortem ultrastructural changes. All observations were carried out under a Jeol JEM 100 CX-II transmission electron microscope in the Department of Electron Microscopy of Oulu University.

Results

The main emphasis in this comparative study was placed on (a) rhabdomeres with their microvilli as places for photopigment and phototransduction and (b) mitochondria as organelles mirroring metabolic activity. At this stage of the investigation, a qualitative assessment of noteworthy changes, accompanied by representative electron micrographs, seemed most appropriate to us.

Within the first 30 min following 'death', photoreceptors from the decapitated flies could be considered normal (Fig. 1), when sections were examined under the electron microscope at magnifications of 3600, 10,000, and 19,000. Each rhabdomere of the photoreceptors displayed neatly arranged and uniformly large subrhabdomeric vesicles, microtubules were visible in the photoreceptor cytoplasm, mitochondria showed the usual cristae and regular dimensions, and central filaments were clearly discernible in transversely-sectioned rhabdom microvilli.

In ether-treated animals 30 min postmortem changes included the disappearance of the subrhabdomeric vesicles, the almost total loss of microtubules, an absence of microvillar central filaments, and washed-out pigment grains. Cell boundaries as well as mitochondria remained clearly identifiable, but the latter had swollen to about twice their normal size and cristae often appeared loose and torn (Fig. 2).

In CO₂-treated material 30 min postmortem subrhabdomeric vesicles appeared enlarged and in a process of drifting proximally (towards the cell centre) away from the edge of the rhabdom. The microvillar central dense core filaments were well preserved and, although affected, the system of microtubules was still present. Mitochondria appeared slightly swollen and the cristae displayed less regular parallel alignments than under normal conditions.

Thirty minutes following an exposure to KCN-vapour subrhabdomeric vesicles were less numerous than in the corresponding CO₂ material. The mitochondrial dimensions were almost normal and cristae were present, but appeared somewhat denser than under normal conditions. Some disruptions of the microvillar dense core filament and the microtubular system were observed, but the changes were minor (Fig. 3).

Three to six hours after decapitation rhabdomeres (Fig. 4) lost their clear delineations with the cytoplasm, showing increasingly closer fusion with and distance to their respective photoreceptor cell bodies (rather than being positioned somewhat like peninsulas projecting outward from the photoreceptor cell into the intercellular space). Mitochondria were enlarged and showed cristae lined with minute electron opaque particles, possibly ribosomes. The microtubular system was gone, but the central dense core filaments within the rhabdom microvilli were still mostly visible in transversely sectioned material. Clear cytoplasmic vesicles

Figs. 1-7. 1. Edge of retinula cell with rhabdomere (R) 15 min after decapitation in air. 2. A similar region with rhabdomere (R) in cell exposed to gaseous ethylether for 30 min. 3. Edge of retinula cell and rhabdomere (R) of cell exposed to KCN-vapour for 5 min. 4. Situation of rhabdomere (R) 6 hr following decapitation in air. 5. Situation of rhabdomere (R) after 5 hr exposure to gaseous ethylether. 6. Situation of rhabdomere (R) after 6 hr exposure to CO₂-gas. 7. Situation of rhabdomere (R) after 3.5 hr exposure to KCN-vapour. Bar: 1 μ m.

Figs. 1-7.

Figs. 8-10. 8. Low power electron micrograph, showing vesicle formation (arrows) at the edge of the rhabdomeres (R) in material exposed for 3.5 hr to KCN-vapour. 9. Rhabdomere-disintegration (R) in material decapitated in air 24 hr postmortem. 10. Rhabdomere-disintegration (R) and endoplasmic reticulum formations (arrow) in material exposed to CO₂-gas for 24 hr. Bar: 1 μ m.

became more conspicuous.

A three to six hours exposure to ether left photoreceptor cells with broken and ruptured membranes, allowing extra- and intracellular contents to mix (Fig. 5). Rhabdomeres displayed breakages of microvilli, but never along the edge with their corresponding retinula cytoplasm. Mitochondria and their cristae were destroyed.

In CO₂-treated animals 3-6 hr postmortem changes (Fig. 6) included an apparent separation (or 'heightened' demarcation) of rhabdomeres from their corresponding cytoplasm. Mitochondria were enlarged, but as with the corresponding material from decapitated flies, contained apparently normal cristae lined with granular, electron opaque material. The development of some intracellular cisternae and rows of endoplasmic reticulum in the photoreceptor cell cytoplasm are indicative of some active processes. Desmosomes between neighbour-

ing cells seemed affected and the dense core filaments in the rhabdom microvilli were no longer identifiable.

In the KCN-animals 3–6 hr postmortem rhabdomere delineations to both extracellular space and corresponding intracellular cytoplasms were still very obvious (Fig. 7). Large numbers of vesicles were very conspicuous along the intracellular edge of the rhabdomere and apparently in the process of moving along some remnant microtubules towards more central regions of the cytoplasm (Fig. 8). Mitochondria were noticeably swollen, but still endowed with parallel cristae. Transversely-sectioned rhabdoms continued to reveal traces of the central microvillar dense core filaments.

After 24 hr (Fig. 9) rhabdoms were much reduced in size; there was little coherence between microvilli, desmosomes were gone, mitochondria enormously swollen, cristae present but disrupted, and ribosomes and/or glycogen granules abundant in localized places of the bodies of the photoreceptor cells of the decapitated animals.

In ether-treated animals all membranes and intracellular organelles had dissolved and apart from foggy shades of grey covering the section to varying degrees, no details were discernible.

In CO₂-treated animals, eyes examined 24 hr later (Fig. 10) displayed swollen rhabdomeres with pigment or otherwise electron opaque grains having taken up positions close to the cytoplasmic (i.e. inner) edges of the rhabdomeres. Large strings of endoplasmic reticulum (rather similar to those found in senescent cells of the bee *Melipona*: DaCruz-Landim and Morelli-Silva de Moraes 1973) were apparent while the swollen mitochondria contained much looser and frequently highly disrupted cristae.

Discussion

Decapitating flies in air or keeping whole flies in CO₂-filled chambers both lead to necrosis through anoxia. However, the progression of cell death as seen by the ultrastructural images of cytoplasmic vesicles, subrhabdomeric cisternae, endoplasmic reticulum, and mitochondria is *clearly not identical* in the two situations, let alone the other experimental conditions. The only unifying character seems that cell deaths proceed from the periphery toward the centre, affecting the rhabdomeres last.

While KCN is undoubtedly a potent cell poison, a total arrest of all intracellular activities did not take place immediately after application. In fact, signs of metabolic activity and certain aspects of intracellular viability were still present in the fly photoreceptor cell several hours after the exposure to KCN. Ethyl ether with its lipid-dissolving capacity caused a very different scenario of necrotic events and presumably led to cell death primarily not because of anoxia, but membrane instability and leakage.

The different reactions of the subrhabdomeric vesicles is of interest in view of the fact that they are thought to store Ca²⁺ and are crucial to the phototransduction process (Minke and Selinger 1996). Their initial diminution and rapid disappearance in ether and KCN-treated flies is irreconcilable with any possible postmortem continuation of photoreception. Their distinct presence in decapitated and even CO₂-material, however, suggests that at least this structural feature, apart from an increase due to swelling, remained relatively unaffected into the post-mortem period. The eventual 'pinching off' of the rhabdomere from its cytoplasmic connection in CO₂- and KCN-treated material might represent a last attempt of the cell to shed its energetically most costly component, namely the assembly of photoreceptive membranes (Tsacopoulos 1995).

The observed loosening of the mitochondrial ultrastructure, an accumulation of coated vesicles and ER in the cytoplasm, the expansion of the system of cisternae, and the loss of microtubules and other cytoskeletal elements, are features shared with visual cells under severe

photo-induced stress (Meyer-Rochow and Horridge 1975, Eguchi and Meyer-Rochow 1983). This similarity is not really surprising, since exposure to light can kill cells (Meyer-Rochow and Tiang 1984).

In conclusion, what the results demonstrate is firstly, that certain aspects of photoreceptor cell function can be 'dissected' by using a range of lethal treatments and that secondly, death by necrosis is not as uniform a process as was generally assumed, but depends on the nature and causes of the accidental death. Consequently, as with the programmed cell deaths, for which Bowen *et al.* (1996) have recently established three subgroups, accidental cell deaths also require the recognition of different subcategories. How many there are and what diagnostic characters are to be used to differentiate them remains to be elucidated and may, in fact, vary from tissue to tissue.

Summary

Cells die either accidental or physiologically programmed deaths. Our results based on ultrastructural changes observed in fly photoreceptors 15 and 30 min, 3, 6 and 24 hr postmortem, demonstrate that death by necrosis is not as uniform a process as had generally been assumed, but depends on the nature and specific causes of the accidental cell demise, which appears to begin peripherally and then moves inward. Consequently, as with programmed cell deaths, death by necrosis also requires the recognition of different sub-categories.

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