ENLARGED SYNAPTIC VESICLES
AS AN EARLY SIGN OF SECONDARY
DEGENERATION IN THE OPTIC NERVE
TERMINALS OF THE PIGEON

M. CUENOD, CLARA SANDRI AND K. AKERT
Brain Research Institute, University of Zurich, Switzerland

SUMMARY

The terminal degeneration of retino-tectal fibres was studied electron microscopically in
the pigeon. Synaptic vesicles seem to undergo systematic changes which can best be observed
in aldehyde-fixed material. Initially (i.e. within 12-24 h) the vesicles begin to swell. The en-
largement is clearly visible after 4 days (40% increase in diameter) and reaches a maximum
at 14 days (100% increase). At the latter stage, the enlargement is almost invariably associated
with the well known opacity of degenerating terminals. In contrast, normal control tissue con-
tains nerve endings with only a few enlarged and no ballooning vesicles. The conclusion seems
warranted that the ballooning of synaptic vesicles is an early sign of terminal degeneration,
it appears to precede vesicular disintegration.

INTRODUCTION

The morphological signs of secondary degenerative alterations in nerve endings as
observed at the electron-microscopic level (for review see Gray & Guillery, 1966)
include an increased electron opacity of the cytoplasm as well as crowding and gradual
disappearance of synaptic vesicles and mitochondria; in many instances, an accumu-
lation of neurofibrillar material has been noted (Gray & Hamlyn, 1962; Guillery,
1965; Walberg & Mugnaini, 1969). In the course of a study of Wallerian degeneration
of optic nerve terminals in the tectum opticum of the pigeon, an early change has been
observed and will be reported here. It concerns the size of synaptic vesicles, whose
enlargement seems to represent the first manifest sign of their deterioration.

METHOD

This study has been performed in 19 pigeons (Columbia livia) of which 17 had their right
retina removed and 2 served as a control. The animals were anaesthetized with Equithesin®
(Jensen-Salsbery) at a dose of 0.25 ml per 100 g. The cornea of the right eyeball and the retina
were carefully removed with a fine suction pipette. Surgery was terminated by leaving a piece
of gelfoam in the eye and by suturing the eyelids tightly.

Twelve hours, 1, 3, 4, 7, 9, 14 and 30 days after the operation the animals were perfused with
4% paraformaldehyde in 0.05 M phosphate buffer (pH 7.4) at room temperature. In 7 cases
the perfusion was made with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at room
temperature. Small pieces (about 1 mm thick) of both optic tecta were post-fixed in glutar-
aldehyde (6.25% in 0.1 M phosphate buffer at room temperature). They were then washed with
phosphate buffer and post-fixed with osmium tetroxide (2% in veronal-acetate buffer and
M. Cuénod, C. Sandri and K. Akert

6.8 % sucrose at 4 °C). Embedding was performed in Epon 812 and the sections were cut with the Porter-Blum microtome. They were mounted on normal or one-hole grids and stained with uranyl acetate and lead hydroxide. Photographs were taken at ×5000 and ×40000 with a Siemens Elmiskop 1.

The outer diameter of synaptic vesicles was measured on plates taken at a magnification of ×40000 using a binocular microscope (× 20) with an ocular micrometer (× 2). All the measurable vesicles in 1 given synaptic ending were systematically taken into account. They were classified according to 2.5-nm steps of diameter. The samples for each of 8 stages of degeneration and the controls consisted of approximately 200 vesicles, which were derived from at least 10 different nerve terminals. Two observers performed the measurements independently on a total of 2557 vesicles. Their results differed by no more than 10 %. Histograms were made and the differences of their mean values and standard deviations were assessed with the t test.

RESULTS

The retinotectal fibres of the pigeon undergo a total crossing in the optic chiasma and, according to the classical histological studies of Ramon y Cajal (1911), terminate between the second and the seventh layers, i.e. roughly in the superficial one-third to one-half of the contralateral optic tectum. Tissue specimens were mainly taken from this region and the observations were systematically compared with the control side. Both axo-dendritic and axo-somatic synapses were encountered. Gray & Hamlyn (1962) have given an excellent description of their fine structure. The present account will be restricted to the problem of synaptic vesicles in normal and degenerating axons. The following description is based on the paraformaldehyde-fixed material, unless otherwise noted.

Control studies

Both optic lobes of 2 intact animals and the ipsilateral tecta of 17 operated animals were examined (Figs. 1, 2 and 9; Table 1). Although nerve endings with flat vesicles were observed, only endings with clear spherical vesicles were selected for this study. The mean diameter of spherical vesicles was 43.2 ± 5.5 nm in the intact animals and 43.5 ± 6.9 nm on the control side of the operated animals.

Degeneration studies

A striking difference of the degenerating tectum is the early occurrence in some synaptic endings of vesicles with a diameter of more than 50 nm. Initially, the growing vesicles have a normal appearance. In the later stages, however, the membranes seem to undergo changes and the centres of the enlarged vesicles tend to be clearer than in the normal material.

At 12 and 24 h survival time a wider range of vesicular diameter is noted and both means are significantly larger (52.5 ± 10.5 and 60.5 ± 12.7 nm respectively) than normal controls (P < 0.001). No other changes are noticeable at the early stages (Fig. 3; Table 1).

At 3 and 4 days (Figs. 1, 10; Table 1) the mean diameter is 63.5 ± 14.0 nm, a value significantly different (P < 0.001) from the control. The enlarged vesicles tend to occupy nearly all the space available in the nerve ending. There might be some
Large vesicles in degenerating synapses

Fig. 1. Histograms of synaptic vesicle diameter in normal and degenerating optic nerve terminals of the tectum. (N = 200). Above: normal animal and control, right tectum of an animal 14 days after ablation of the right retina. Below: degenerating, left tectum, at 4 days and 14 days after ablation of the right retina.

Table 1. Diameter of synaptic vesicles in normal and degenerating optic nerve terminals of the pigeon optic tectum

<table>
<thead>
<tr>
<th>Survival time (days)</th>
<th>Tectum</th>
<th>Fixation</th>
<th>Observer</th>
<th>N</th>
<th>X</th>
<th>s.d.</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>L</td>
<td>P</td>
<td>A</td>
<td>200</td>
<td>43.2</td>
<td>5.5</td>
<td>0.4</td>
</tr>
<tr>
<td>(normal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>R</td>
<td>P</td>
<td>A</td>
<td>200</td>
<td>43.5</td>
<td>6.9</td>
<td>0.5</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>L</td>
<td>P</td>
<td>A</td>
<td>200</td>
<td>52.5</td>
<td>10.5</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>L</td>
<td>P</td>
<td>B</td>
<td>186</td>
<td>60.5</td>
<td>12.7</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>L</td>
<td>P</td>
<td>A</td>
<td>200</td>
<td>63.5</td>
<td>14.0</td>
<td>1.0</td>
</tr>
<tr>
<td>14</td>
<td>L</td>
<td>P</td>
<td>A</td>
<td>200</td>
<td>66.4</td>
<td>16.9</td>
<td>1.2</td>
</tr>
<tr>
<td>30</td>
<td>L</td>
<td>G</td>
<td>A</td>
<td>203</td>
<td>55.9</td>
<td>10.3</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>175</td>
<td>60.5</td>
<td>10.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

The right retina was ablated. Measurements of 2 observers (A and B) were computed. N = Sample number; X = mean diameter, nm; s.d. = standard deviation; s.e. = standard error; L = left tectum; R = right tectum; P = Paraformaldehyde/OsO₄; G = Glutaraldehyde/OsO₄.
alterations of the mitochondria, but no change is seen in the synaptic cleft or in the post-synaptic formations.

At 7 days (Fig. 6; Table 1) a further increase in size (68.1 ± 16.0 nm mean diameter) and a reduction in the number of synaptic vesicles is noted.

At 14 days (Figs. 1, 4, 5 and 7; Table 1) the vesicles are still larger, their mean diameter being 80.2 ± 18.2 nm, and the largest reaching 150.0 nm. These values differ significantly \( P < 0.001 \) from those obtained at 4 days (Table 1). At this time the reduction in vesicular number is clearly visible, and the remaining vesicles appear against an electron-dense cytoplasmic matrix (Figs. 6–8). Their centres often appear to be less electron-dense than normally (Figs. 4, 5, 7). One also notices at the 14 day-stage that the ‘tramline’ pattern of unit membranes of the vesicles seems less clearly defined: the osmiophilic membrane leaflets of the ballooning vesicles (Figs. 4, 5) seem to be thinner and closer together. In addition, the mitochondria have usually deteriorated and glial processes begin to surround the degenerating terminals.

At 30 days (Fig. 8; Table 1) the degenerating endings are almost solidly electron-opaque and only exceptionally can organelles be discerned. The post-synaptic membrane apposition remains the sole criterion of a synapse in these instances. Eventually, all degenerated terminals seem to be engulfed by glia cells and the contact with post-synaptic structures is obscured.

In animals perfused with glutaraldehyde instead of paraformaldehyde solution one may observe analogous changes but to a somewhat lesser degree. Again, the range and peak of vesicular diameters are increased significantly beyond normal control values (Figs. 10, 11; Table 1). However, fewer extreme stages of ballooning are observed. On the other hand, it seems that this fixative tends to provide better preservation of neurofilaments. Thus, the accumulation of fibrillar masses as first described by Gray & Hamlyn (1962) is more frequently encountered in this material (Fig. 11).

DISCUSSION

The present findings strongly suggest that the enlargement of synaptic vesicles is among the first morphological signs of terminal degeneration. The swelling is measurable as early as 12 h and becomes clearly evident within the first post-operative days, certainly long before the opacity of cytoplasmic matrix develops. After 1 week of degeneration the number of synaptic vesicles decreases noticeably. Therefore, it may be assumed that the ballooning vesicles burst into membranous pieces which disappear within the dark matrix of the terminal together with mitochondrial debris. This notion is consistent with Walberg's (1965) statement (p. 209): 'While synaptic vesicles are readily recognized at the 2 and 4-day stages their individuality is usually lost by the seventh day. This is probably due to a disintegration of the vesicles with a rupture of their membrane'.

The observation of vesicular enlargement is not entirely novel. Colonnier (1964) and Smith, Hudgens & O'Leary (1966) have described and demonstrated some degree of swelling and others have shown enlarged vesicles in their illustrations (Gray, 1964; Walberg, 1964, 1965; Conradi & Skoglund, 1968; Raisman, 1969).
Hamori & Tömöl (1966) noted on the second and third day of degeneration that optic nerve terminals in the cat lateral geniculate body contain synaptic vesicles of more irregular size. But no one has made a concentrated effort to study the vesicular changes, even though agreement exists that synaptic vesicles appear initially intact and begin to disappear after about 1 week’s degeneration (Alksne, Blackstad, Walberg & White, 1966; Gray & Guillery, 1966). The ballooning of vesicles is best seen in material prefixed with paraformaldehyde and is somewhat less conspicuous (but still noticeable) in glutaraldehyde-prefixed tissue. Gray & Hamlyn (1962), using the same species and the same anatomical system but fixing with osmium tetroxide, failed to detect enlarged vesicles in degenerated endings. This discrepancy is of considerable interest, since it suggests that the enlargement of synaptic vesicles is closely related to the effects of aldehyde fixation.

The second decisive factor is the time course of degeneration, which may vary with respect to species and localization in the nervous system (Alksne et al. 1966). For instance, in the rat cortex it might be very difficult to detect the ballooning stage of degenerating synaptic vesicles since Colonnier (1964) found that full-blown degeneration, in which the synaptic vesicles no longer appear as discrete structures, may occur as early as 24 h after making the lesion. A similar situation was found at the mammalian motor endplate by Nickel & Waser (1968). The avian optic system takes a slower time course of degeneration and offers better opportunities for the study of certain details.

The significance of the vesicular alteration is not yet known. Possibly the vesicular membrane undergoes structural changes accompanied by alterations of selective permeability. The ballooning is probably the result of increased fluid uptake. However, the vesicular membranes seem initially intact and the appearance after 2 weeks (blurring and thinning of the ‘tramlines’) seems more likely to be the effect of ballooning than its cause. Perhaps the primary change concerns the osmolarity of the axoplasm. Studies on axoplasmic transport of proteins in pigeon optic nerve have revealed a migration of material in less than 12 h from the retinal ganglion cells to their tectal synaptic endings (Cuenod & Schonbach, 1969). The lack of this renewal of material after retinal ablation may be responsible for changes of axoplasmic concentration in the degenerating terminals.

This work was carried out with the aid of Grants No 4806 and 4356 from the Swiss National Foundation for Scientific Research and the Hartmann-Müller Foundation for Medical Research in Zurich.

The statistical analysis was made by U. R. Wyss. A. Fäh, F. Robert and R. Meier assisted in the surgical procedures and post-operative care of the animals. All this help is gratefully acknowledged.
REFERENCES


(Received 27 June 1969)
Large vesicles in degenerating synapses

Figs. 2–5. Enlargement of synaptic vesicles during Wallerian degeneration of retinotectal nerve endings in the pigeon. Fig. 2, normal control; Fig. 3, 24 h after removal of contralateral eye; Figs. 4 and 5, 14 days survival (m = mitochondria; pr, presynaptic endings, sv, synaptic vesicles; arrow, site of synapses). Initial magnification, × 40 000. Paraformaldehyde perfusion; post-fixation with glutaraldehyde/OsO₄.
Figs. 6–8. Increased electron density of degenerating boutons terminaux. Note the presence of enlarged vesicles in Figs. 6 and 7. Fig. 6, 7 days survival; Fig. 7, 14 days survival; Fig. 8, 30 days survival at which stage the membranous structures are hard to identify (arrows indicate synaptic sites; pr, presynaptic endings; sv, synaptic vesicles). Initial magnification, ×40000. Paraformaldehyde perfusion; post-fixation with glutaraldehyde/OsO₄.
Figs. 9–11. Enlarged synaptic vesicles at various stages of degeneration as seen in glutaraldehyde-fixed material. Fig. 9, normal control; Fig. 10, 3 days survival; Fig. 11, 9 days survival. Note the fibrillar hypertrophy in Fig. 11 (ax, axon; nf, neurofilaments; pr, presynaptic endings; arrows indicate synaptic sites). Note synapses en passage in Fig. 11. Initial magnification, ×40,000. Glutaraldehyde perfusion, post-fixation with OsO₄.