Cytochemical Studies on Golgi Apparatus, GERL, and Lysosomes in Neurons of Dorsal Root Ganglia in Mice

(enzyme localizations/electron microscopy/cytology/beige mouse/acid phosphatase latency)

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ABSTRACT Cytochemically demonstrable thiamine pyrophosphatase activity is present in the innermost Golgi element in both small and large neurons of the dorsal root ganglia in CF1, C57 black, and C57 beige mice, thus resembling the neurons of rat dorsal root ganglia. The localization of acid phosphatase (EC 3.1.3.2) activity in the large neurons of dorsal root ganglia in these mice is also similar to that in rats; it is not demonstrable in Golgi elements but is present in GERL and in three types of lysosomes apparently derived from GERL. However, the small neurons of the mouse differ from those of the rat in showing acid phosphatase activity in all elements of the Golgi apparatus. In the mouse neurons the acid phosphatase activity of residual bodies is “latent,” i.e., it is not demonstrable in well-preserved cells.

The three organelles dealt with in this communication are closely related structurally, and probably functionally: (a) the Golgi apparatus, (b) GERL—a specialized region of smooth endoplasmic reticulum that is located at the “trans” (1) face of the stack of Golgi elements and that possesses cytochemically demonstrable acid hydrolase activities, and (c) lysosomes (2–4). In an early account (5), small neurons of dorsal root ganglia in 13-month CF1 mice were found to display acid phosphatase activity in the three organelles, including all “elements” (2) of the Golgi apparatus. “It remains to be established,” we wrote, “if this difference from the rat findings reflects the difference in age . . . or species” (5).

Our present observations establish the difference to be a species difference between mouse and rat. Although acid phosphatase activity is demonstrable in the Golgi apparatus of the small neurons, the large neurons in the mouse dorsal root ganglia fail to show this activity. Another interesting difference between dorsal root neurons of mouse and rat is that in the mouse the type of lysosome known as residual body (3) does not reveal its acid phosphatase activity in well-preserved cells, i.e., this activity is “latent” as in lysosomes carefully isolated from liver homogenates (6). In contrast, two other types of lysosomes, coated vesicles and autophagic vacuoles of the type-2 variety (3), show acid phosphatase activity even when the residual bodies fail to do so.

MATERIALS AND METHODS

Three strains of mice were studied: CF2, purchased from Carworth Farms, Wilmington, Mass., homozygous C57 black/6J, and its beige mutant (bg/bg), both obtained from the Jackson Laboratories, Bar Harbor, Me. Animals of both sexes were used; they ranged in age from 8 to 26 weeks.

As with rats, good preservation of neurons requires fixation by perfusion. Similar results were obtained by perfusion with 2.5% glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.)–0.1 M cacodylate buffer, pH 7.4 with 0.05% CaCl2 (7) or with 2.5% glutaraldehyde–2% formaldehyde (prepared from paraformaldehyde purchased from Fisher Scientific Co.)–0.09 M cacodylate, pH 7.4, with 0.025% CaCl2 (8). The mice were anesthetized by ether, and cold (4°C) fixative was perfused via the heart and aortic arch for 10 min. This was followed by immersion fixation for 120 min in the same fixative as used for the perfusion.

Frozen sections, 10 μm in thickness, were cut on a Sartorius microtome and incubated for either thiamine pyrophosphatase or acid phosphatase [EC 3.1.3.2; orthophosphoric-monoester phosphohydrolase (acid optimum)] activity. These were examined by light microscopy. The results, consistent with those of electron microscopy, will not be illustrated. For electron microscopy, 30 to 40-μm nonfrozen sections were cut into 7.5% sucrose with the Sorvall TC2 Smith-Farquhar tissue sectioner. The sections were separated from the agar and incubated, freely floating, in thiamine pyrophosphatase or acid phosphatase medium at 37°C with slow shaking in the Dubnoff water bath.

The medium for thiamine pyrophosphatase was that of Novikoff and Goldfischer (9). The ingredients are 25 mg of thiamine pyrophosphate (Sigma Chemical Co.); 7 ml of distilled water; 10 ml of 0.2 M Tris-maleate buffer, pH 7.2; 5 ml of 0.025 M manganese chloride; 3 ml of 1% lead nitrate; and 1.25 g of sucrose. The medium is filtered after 5–10 min, and renewed each 30 min, when longer incubations are used.

Acid phosphatase activity was always tested with CMP and β-glycerophosphate (both from Sigma Chemical Co.). The ingredients of the CMP medium are 25 mg of CMP, 12 ml of distilled water; 10 ml of 0.05 M acetate buffer, pH 5.0; 3 ml of 1% lead nitrate; and 1.25 g of sucrose. The medium is filtered after 5–10 min. The β-glycerophosphate medium is prepared by adding 2.0 ml of β-glycerophosphate to 18.2 ml of 0.05 M acetate buffer, pH 5, and then slowly adding 2.0 ml 1.2% lead nitrate. After the medium is incubated at 37°C for 30 min and filtered, 1.10 g of sucrose are added.

When the residual bodies were found to be acid-phosphatase-negative in well-fixed perikarya, three methods were used to determine if this were due to latency: (a) freezing and thawing, five times, prior to sectioning; (b) prolonged incubation (3 hr); and (c) immersion fixation, as above, without initial perfusion. Only the last procedure proved effective in demonstrating latency, although the first two have been found.

Abbreviations: ER, endoplasmic reticulum.

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RESULTS

No difference is evident among the three mouse strains studied with respect to the organelles to be described, save for the large size of the residual body-type lysosomes in the beige mouse. Nor are differences seen between males and females, or in mice of different ages. In all mice, virus-like particles are encountered in the endoplasmic reticulum (ER) occasionally (V in Fig. 10).

The structural relations among rough ER ("Nissl substance") (N in Figs. 2, 3, and 10); smooth ER in the form of "transitional vesicles" and "transitional sheets" (arrows in

by others (10–13) to be useful in overcoming the latency of leukocyte lysosomes.

FIGS. 1–4. Portions of neurons from dorsal root ganglia. Labels are explained in text. Fig. 1. Small neuron, not incubated, female C57 black, 12 weeks. ×22,000. Fig. 2. Small neuron (above) and large neuron (below), incubated with β-glycerophosphate 33 min, female CF1, 8 weeks. ×15,000. Fig. 3. Small neuron, incubated with β-glycerophosphate 29 min, female C57 black, 12 weeks. ×7000. Fig. 4. Small neuron, incubated with β-glycerophosphate 29 min, female CF1, 8 weeks. ×30,000. All ganglia were fixed by perfusion and immersion in glutaraldehyde-formaldehyde.
Figs. 5–8. Portions of neurons from dorsal root ganglia. Labels are explained in text. Fig. 5. Small neuron, incubated with β-glycerophosphate 34 min, female C57 black, 12 weeks. X14,500. Fig. 6. Large neuron, incubated with CMP 38 min, male C57 black, 26 weeks. X13,500. Fig. 7. Small neuron, incubated with CMP, 37 min, male C57, 8 weeks. X32,000. Fig. 8. Large neuron, incubated with CMP, 15 min, male C57 beige, 26 weeks. X8000. Fixation: Figs. 5 and 7, glutaraldehyde-formaldehyde; Fig. 6, glutaraldehyde-all by perfusion and immersion. Fig. 8, glutaraldehyde by immersion only.

Figs. 1 and 10) directed towards the outermost [cis (1)] element of the Golgi apparatus (G in Figs. 1–7 and 10, and arrows in Fig. 9); and GERL (GE in Figs. 1–7 and 10) are similar to those described in the small neurons of the rat dorsal root ganglia, as drawn diagrammatically in Fig. 40 of ref. 2. In both small and large neurons of the mouse, the Golgi apparatus consists of five or six elements, roughly evenly spaced (Figs. 1, 2, 4–6, 9, and 10). GERL (Figs. 1–7 and 10) is separated from the trans element of the Golgi apparatus by regions of variable size inside of which vesicles or tubules are often seen (Figs. 1, 2, 7, and 10). As in small neurons of the rat (2), GERL consists of cisternal and tubular portions. The tubules show fenestrations (Figs. 4 and 5). However, these fenestrations are less regularly arranged than the hexagonal fenestrations of the trans element of the Golgi apparatus (Fig. 9). The lysosomes related to GERL will be considered in the paragraph describing localization of acid phosphatase medium activity.

**Thiamine Pyrophosphatase Activity.** In the perikarya of both small and large neurons, thiamine pyrophosphatase activity is restricted to the Golgi apparatus, and only the element at the trans face shows such activity. This element is composed exclusively of tubules arranged in a geometrically regular hexagonal array (Fig. 9). Within the hexagons thiamine pyrophosphatase-negative tubules are found, which in transverse section appear as vesicles (inset, Fig. 9), as in the rat small neurons (2).

**Acid Phosphatase Activity.** The small neurons display marked acid phosphatase activity in GERL, with either β-glycerophosphate or CMP as substrate (Figs. 2–5 and 7). They also display high acid phosphatase activity in all elements of the Golgi apparatus (Figs. 2–5 and 7), with reaction product accumulating more rapidly with β-glycerophosphate than with CMP. In the large neurons GERL is smaller relative to the Golgi apparatus than in the small neurons and its level of acid phosphatase activity appears to be lower, although this was not thoroughly investigated. The most striking contrast between large and small neurons is that all Golgi elements are without demonstrable acid phosphatase activity in the large neurons.
Three types of lysosomes are abundant in both small and large neurons: coated vesicles (C in Figs. 1, 2, and 10); autophagic vacuoles, type 2 [labeled A in Figs. 1–5, 7, 9, and 10 (the three structures to the sides of A in Fig. 2 probably are such vacuoles in which the inner tubules have been obscured by reaction product)]; and residual bodies (R in Figs. 1–3, 5–7, 9, and 10). These lysosomes have been discussed in a recent review (3). It is interesting to note that, in contrast to the hepatocytes of beige mice described by Essner and Oliver (14), GERL in the dorsal root ganglia neurons of beige mice is not larger than in black mice although the residual bodies are, as in the hepatocytes, very much larger (Figs. 9 and 10).

Coated vesicles are attached to or lie adjacent to GERL, as if arising from GERL (Figs. 1, 2, and 10). The vesicles show the same abundance of acid phosphatase reaction product as does GERL in the small neurons (Fig. 2), but in some large neurons they are less reactive. The coated vesicles thus resemble those of the rat neurons (2).

Most autophagic vacuoles, type 2, have a structure similar to those of the rat neurons and probably derive from GERL in a manner like that suggested for the rat (2, 3) (Figs. 1, 2, 7, 9, and 10). Others, however, appear to form by GERL membranes sequestering areas of cytosol, sometimes along with formed structures (Figs. 1, 4, and 6). The autophagic vacuoles, type 2, like the coated vesicles, show the same abundant acid phosphatase reaction product as GERL does in the small neurons (Figs. 2–5 and 7). In the large neurons the level of activity is considerably lower.

In both large and small neurons, lysosomes with the typical fine structure of residual bodies (R) are numerous. As seen most clearly in Figs. 1, 7, and 10, the residual body (R) is delimited by a relatively thick tripartite membrane, often revealing an underlying clear area ("halo"), and it contains electron-opaque grains, membranous arrays, or, particularly in the beige mouse (see ref. 14), electron-lucent lipid-like areas (marked L in Figs. 9 and 10). The striking feature of the residual bodies in these mouse neurons is that they demonstrate either no acid phosphatase activity (Figs. 2, 3, 5, and 7) or very faint accumulations of reaction product (uppermost bodies marked R in Figs. 2 and 7). However, this reflects the latency of the hydrolase. In ganglia fixed by immersion only, the fine structure of many neurons is poorly preserved, as witnessed, e.g., by ballooned empty-looking mitochondria (M in Fig. 8). In such neurons all residual bodies show much acid phosphatase reaction product (Fig. 8).

Three additional features of the acid phosphatase activity of these neurons should be noted: (a) Sodium fluoride (1 mM) totally inhibits its activity in all sites—GERL, lysosomes, and Golgi apparatus. (b) In neither small nor large neurons is activity seen in the ER; (c) No evidence was encountered of acid phosphatase-positive vesicles apparently arising from Golgi elements, even in the small neurons.

Large Neurons of Rat Dorsal Root Ganglia. The large neurons have not been included in our published descriptions (2, 5). To permit fuller evaluation of the mouse findings, the rat observations† are summarized here. Although not seen in light micrographs (5), GERL is present and shows high levels of acid phosphatase activity. The Golgi apparatus reveals no such activity. Also as in rat small neurons (2) only the trans element of the Golgi apparatus shows thiamine pyrophosphate activity, and this element consists entirely of tubules in hexagonal array. Thus, in these regards the large neurons of the rat are like the large neurons of the mouse.

DISCUSSION

Small and large neurons in dorsal root ganglia differ from each other in histochemical reactions as well as in cytological

† P. M. Novikoff, unpublished observations.
features and probably in function (see p. 310 in ref. 5). The present study adds a striking cytochemical difference in mice, the small neurons displaying high levels of acid phosphatase activity in the Golgi apparatus and the large ones showing no activity at all under our conditions of fixation and incubation. Whatever its functional significance proves to be, this difference might be presently exploited to study, by experimental manipulation, unsettled questions regarding lysosomal “packaging” and “membrane flow” in the Golgi apparatus.

For example, can the Golgi apparatus under some circumstances assume part of GERL’s functions in packaging of acid hydrolases and other materials into lysosomes? Decker (4) has studied lateral motor column neurons of the frog tadpole. At a given period of development, if some neurons presumably fail to make peripheral connections, they undergo degeneration. At this time the neurons “exhibit acid hydrolase activity within GERL elements and, possibly, some activity within Golgi sacules” (4), whereas at other times only GERL shows hydrolase activities. In these degenerating neurons, acid phosphatase activity does not become demonstrable in the ER as it does in HeLa cells after arginine deprivation or ultraviolet radiation, presumably because in HeLa degeneration the level of activity becomes sufficiently high in the ER so that the relatively insensitive cytochemical procedure can reveal its presence (15).

An earlier publication (2) discussed the difficulties in assuming that cis elements move downward to become trans elements (pp. 876–878). This type of “membrane flow” may better be studied in the mouse where one class of neuron has demonstrable acid phosphatase activity and the other class does not. This difference has not been encountered in other mammals.

There has been considerable discussion that, with aging, residual bodies may lose hydrolase activities and become “post-lysosomes” in the terminology of de Duve and Wattiaux (16). When recently discussing the hazard of using cytochemical procedures for demonstrating such loss, we wrote, “However, the possibility should be borne in mind that one or more ingredients of the incubation medium, possibly the lead ions, cannot permeate the delimiting membrane” (ref. 3, p. 28). Whatever the mechanism involved, the observations described in this communication add neurons of mice dorsal root ganglion to leukocytes (10–13) as cells in which lysosomes display latency in cytochemical reactions.

An early suggestion from this laboratory has found widespread adoption, namely that Golgi vesicles, i.e., small buds from elements of the Golgi apparatus, are the so-called primary lysosomes (16). Yet in this study, as in studies on several other cell types, evidence for this suggestion was not found, and the acid phosphatase-rich coated vesicles derived from GERL probably function in this capacity. However, in leukocytes and possibly other cell types, Golgi elements do produce primary lysosomes (17).

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