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Alterations in the intermediate layer of goldfish meninges during adaptation to darkness

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ABSTRACT

The morphological changes in the intermediate endomeningeal layer of the goldfish brain during light and dark adaptation were studied by freeze-fracture electron microscopy. During the different stages of adaptation no significant changes were found in the density of intramembrane particles and nuclear pores in these cells. The density of plasmalemmal vesicles in the meningocyte surface increased in the groups maintained in the dark for 48 and 72 h (maximum) and then decreased in the group maintained for 96 h in the dark to a basal level. There were also morphological changes in the junctional complexes. At the upper cell membranes (in contact with the outer layer) of meningocytes in the group maintained in the dark for 48 h, we found an increase in the surface occupied by gap junctions. In addition, gap junctions were absent in the lateral membranes of meningocytes from animals maintained in the dark for 72 h. The morphology of gap junctions in the group maintained in the dark for 96 h was similar to that of the control group. These results suggest that the cells of the teleost intermediate endomeningeal layer undergo important changes in activity during adaptative experiments.

Key words: Freeze-fracture; membrane structure; intercellular junctions; tight junctions; gap junctions; ependymin.

INTRODUCTION

The teleost endomeninx comprises 3 distinct layers attached to the brain: named outer, intermediate and inner layers according to their proximity to the brain (Momose et al. 1988; Caruncho et al. 1993). A 4th layer—the ectomeninx—is composed of mucous tissue, collagen fibres and blood vessels (Caruncho et al. 1993). The cells of the intermediate endomeningeal layer are joined by well developed junctional complexes that are the morphological basis of the meningeal diffusion barrier. These cells exhibit morphological characteristics similar to endothelial cells and it is believed that they participate in active transcellular transport (Momose et al. 1988; Caruncho et al. 1993).

Our aim in this work was to investigate whether adaptation to darkness induces changes in the morphology of the intermediate meningeal cells that are possibly related to the production of ependymins (a class of glycoproteins expressed in fish meninges that show a high turnover level during learning experiments and play an important role in the development of long-term memory in teleosts) (Shashoua, 1981, 1982, 1985; Konigstorfer et al. 1990; Hoffmann, 1992; Schmidt et al. 1992). To this end we designed experiments that involved the adaptation of goldfish from a regime of equal photoperiod (12 h light/12 h darkness) to constant darkness. Habituation is one of the simplest learning experiences. Dark adaptation in teleosts has been shown to induce a substantial anatomical reorganisation in retinal horizontal cells, changes that correlate with variations in their physiological response to light (Weiler et al. 1988; Weiler & Schultz, 1993). Recently it has been demonstrated that these changes are due to the activation of ionotropic AMPA receptors (Weiler & Schultz, 1993). The meninges are

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the site of production of ependymins, proteins that display a high level of turnover during learning experiments. We therefore hypothesise that there may be important changes in activity in meningeal cells during light and dark adaptation. We report here the ultrastructural changes in the intermediate layer meningocytes as shown by freeze-fracture electron microscopy.

MATERIALS AND METHODS

Forty goldfish ~ 8 cm in length were divided in 1 control group and 4 experimental groups of 8 animals each. The experimental groups were maintained in the dark before being killed at 24, 48, 72, and 96 h respectively, while the control fish were kept in a constant photoperiod of 12 h light/12 h darkness. The animals of each experimental group were processed together with 2 animals from the control group.

Fish were killed by decapitation under anaesthesia with MS 222 (Sigma Chemical Co.) and the meninges were stripped off the brain, fixed in a solution of 0.5% glutaraldehyde and 4% paraformaldehyde, glycerinated and frozen in nitrogen slush. Freeze-fracture of the meninges was performed as described previously (Caruncho et al. 1990). The replicas were observed and photographed in a Phillips EM410 transmission electron microscope.

Plasmalemmal vesicles and nuclear pores were counted in squares of a grid that was superimposed on electron micrographs at ×20000 final magnification, so that each square corresponded to 1 μm^2. Counting was made in 5 different replicas from each group (the number of cells analysed was 5 per replica to total a number (n) of 25 cells analysed per group). The total area of surfaces analysed was ~ 150 μm^2 per group for the measurement of density of plasmalemmal vesicles and 50 μm^2 per group for the density of intramembranous particles (IMPs) and nuclear pores. Corrections for curved surfaces (i.e. nucleus) were made according to Garcia-Segura & Perrelet (1984). For statistical comparison of data a one-way ANOVA test ($P < 0.05$) (Daniel, 1991) was used.

RESULTS

To assess possible changes that occur in the intermediate layer cells of the goldfish meninges during adaptation to darkness, we analysed the densities of plasmalemmal vesicles and nuclear pores. In addition we studied the morphology of the intercellular junctions. The quantitative results are summarised in Table 1 and a summary of the general anatomical characteristics of teleost meninges is given in Table 2.

The densities of IMPs were unchanged during the different stages of the experiment. The values were similar to those of the control group: about 750 IMPs μm^{-2} in the P face and 200 IMPs μm^{-2} in the E face, with no significant differences in densities between the upper (in contact with the outer layer) and lower (in contact with the inner layer) plasma membranes.

In fish maintained in the dark for 24 h before killing there were no significant differences in the densities of plasmalemmal vesicles in comparison with the control group (5.8 ± 1.2 μm^{-2} and 6.5 ± 1.3 μm^{-2} respectively). When fish were maintained in the dark for 48 h before killing, the density of plasmalemmal vesicles was significantly higher at 9.8 ± 1.6 μm^{-2}. In animals from the group maintained in darkness for 72 h the density of plasmalemmal vesicles in the intermediate layer meningocytes (20.7 ± 4.3 μm^{-2}) was significantly increased in comparison with all other groups (Table 1). When fish were maintained in darkness for 96 h the number of plasmalemmal vesicles returned to the basal level (5.9 ± 0.9 μm^{-2}).

In all groups the densities of plasmalemmal vesicles were similar in the upper and lower membranes. Some plasmalemmal vesicles are indicated by squares in Figures 1 (control group), 2*a* and *b* (24 h in darkness), 3*a* and *b* (48 h in darkness), 4*a* and *b* (72 h in darkness) and 5*a* (96 h in darkness). There were no

Table 1. *Changes in density of caveolae and nuclear pores in the intermediate layer meningocytes during adaptation to darkness*

Adaptation to darkness	Caveolae μm^{-2}	Nuclear pores μm^{-2}
24 h in darkness	5.8 ± 1.2	7.6 ± 1.9
48 h in darkness	9.8 ± 1.6*	6.9 ± 1.2
72 h in darkness	20.7 ± 4.3*	8.3 ± 1.9
96 h in darkness	5.9 ± 0.9	6.5 ± 1.1
Control group	6.5 ± 1.3	7.2 ± 1.8

* Significantly different ($P < 0.05$) one-way ANOVA test.

Table 2. *Morphological characteristics of teleost meninges*

Cranium
Ectomeninx: mucous tissue, collagen fibres, blood vessels
Leptomeninx:
outer layer: 2–6 layers of flattened cells, few intercellular spaces
intermediate layer: single layer of elongated cells with well developed junctional complexes
inner layer: spindle-shaped cells, abundant intercellular spaces, collagen fibres, blood vessels
Terminal glia

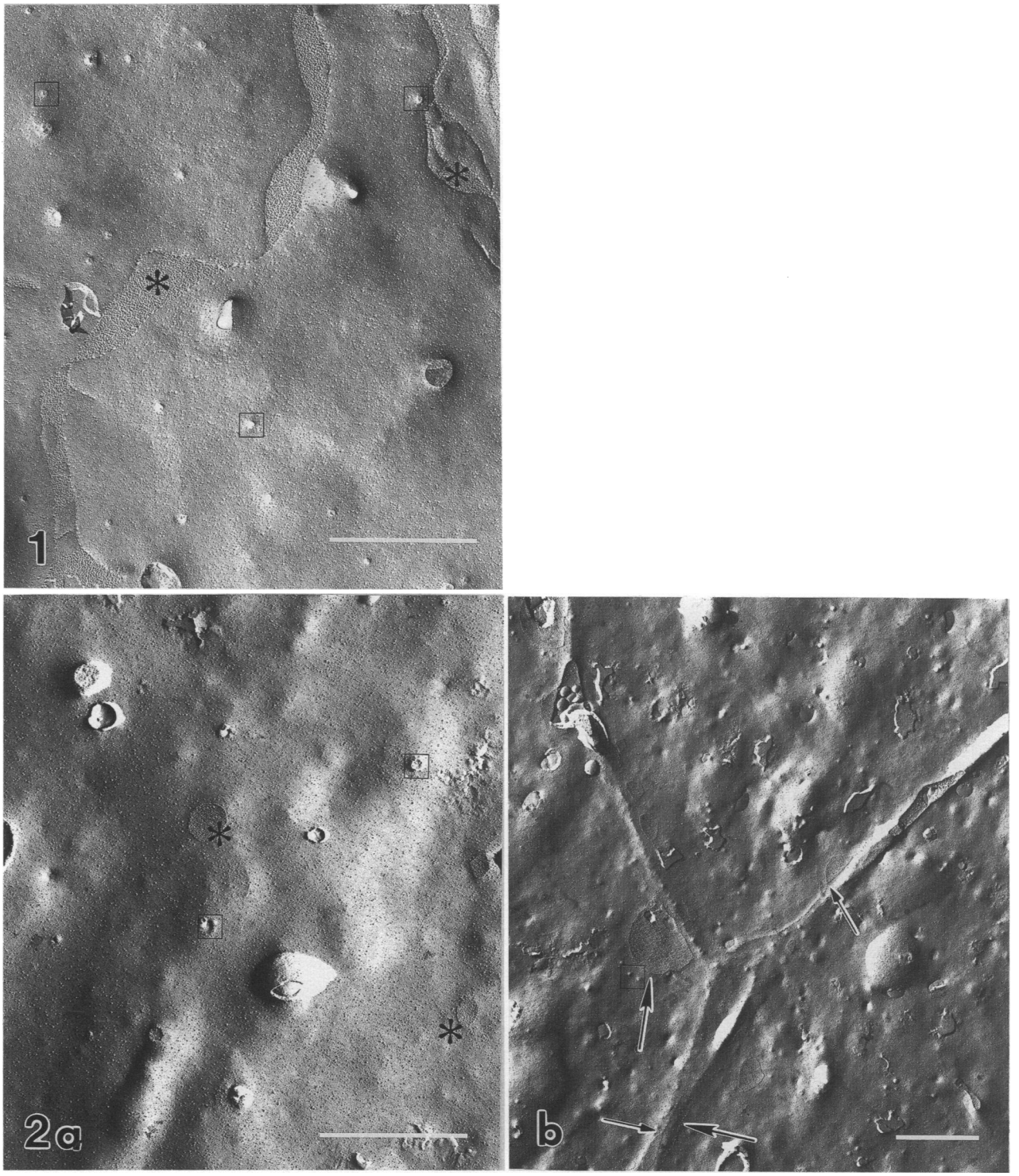

Fig. 1. Freeze-fracture replica of the P face of the lower membrane of an intermediate layer meningocyte from the control group. Some plasmalemmal vesicles are identified by squares and gap junctions by asterisks. Bar, 1 μm. In this and all succeeding figures, the direction of shadowing is from bottom to top.

Fig. 2. Freeze-fracture replicas of brain meninges from fish maintained in the dark for 24 h. (*a*) P face of the upper membrane of an intermediate layer meningocyte. Asterisks indicate gap junctions and squares plasmalemmal vesicles. Bar, 1 μm. (*b*) P face of a lower membrane of an intermediate layer meningocyte. The large arrows identify gap and tight junctions that are separated while the small arrow shows gap and tight junctions in association. Squares outline some plasmalemmal vesicles. Bar, 1 μm.

significant differences in densities of nuclear pores in the meningocytes from the different groups (see Table 1).

We also examined the morphological changes in the intercellular junctional complexes. In the control group these complexes mainly consisted of rounded or

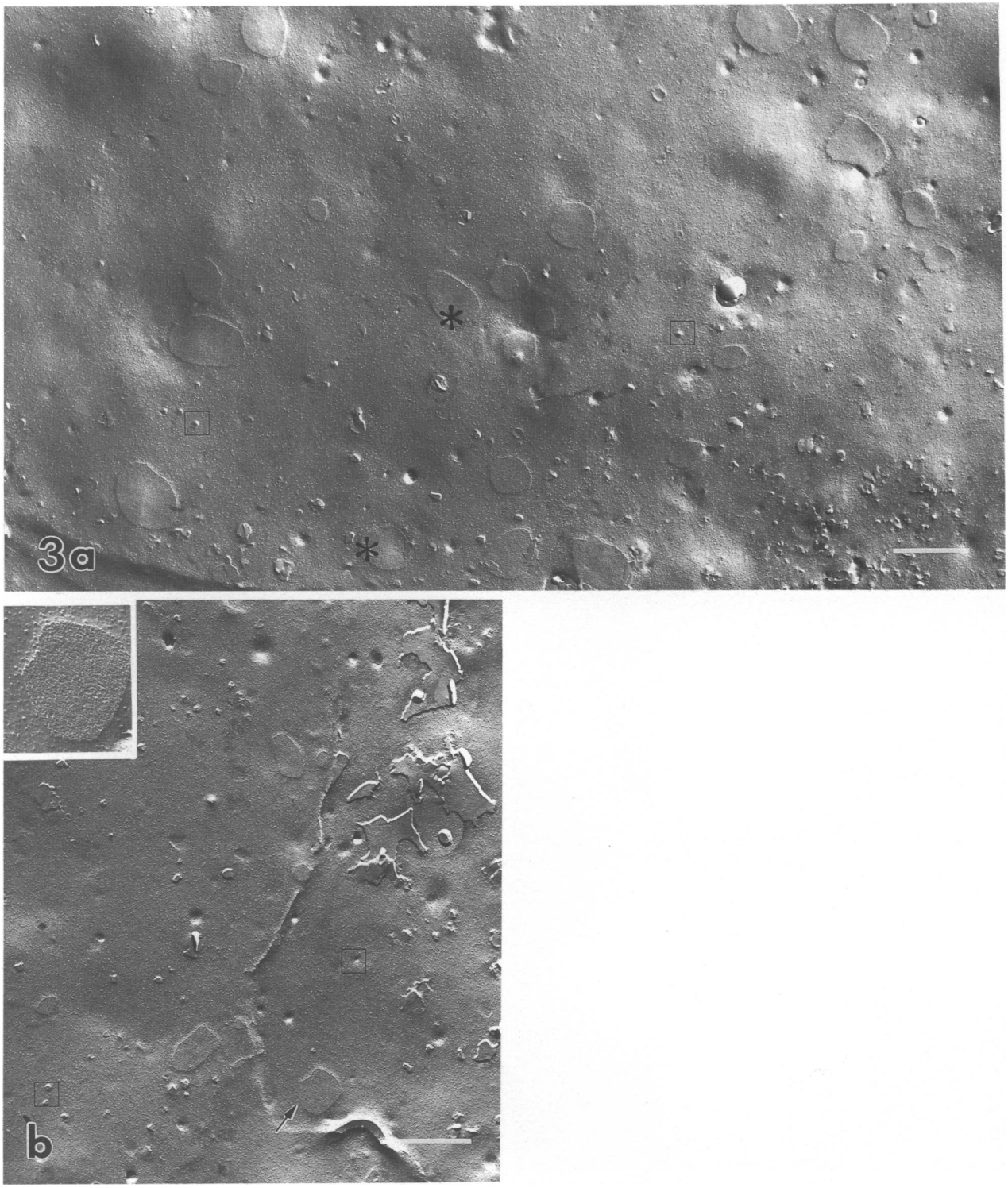

Fig. 3. Freeze-fracture replicas of brain meninges from fish maintained in the dark for 48 h. (*a*) P face of the upper membrane of an intermediate layer meningocyte. Note abundance of macular gap junctions (asterisks) that have cleaved with the E face of the adjacent cell. Squares indicate caveolae. Bar, 1 μm. (*b*) P face of the lower membrane of an intermediate layer meningocyte. Squares outline caveolae. Bar, 1 μm. Inset: Higher magnification of the E face of a gap junction (small arrow) showing a crystalline arrangement of the connections. ×40000.

elliptical gap junctions in the upper plasma membrane (asterisks in Fig. 1) and gap junctions associated with tight junctions and desmosomes in the lower membranes. In lateral membranes there was a hexagonal network of tight junctions associated with gap junctions and desmosomes (not illustrated).

In the group of fish maintained in the dark for 24 h the upper plasma membrane of the meningocytes

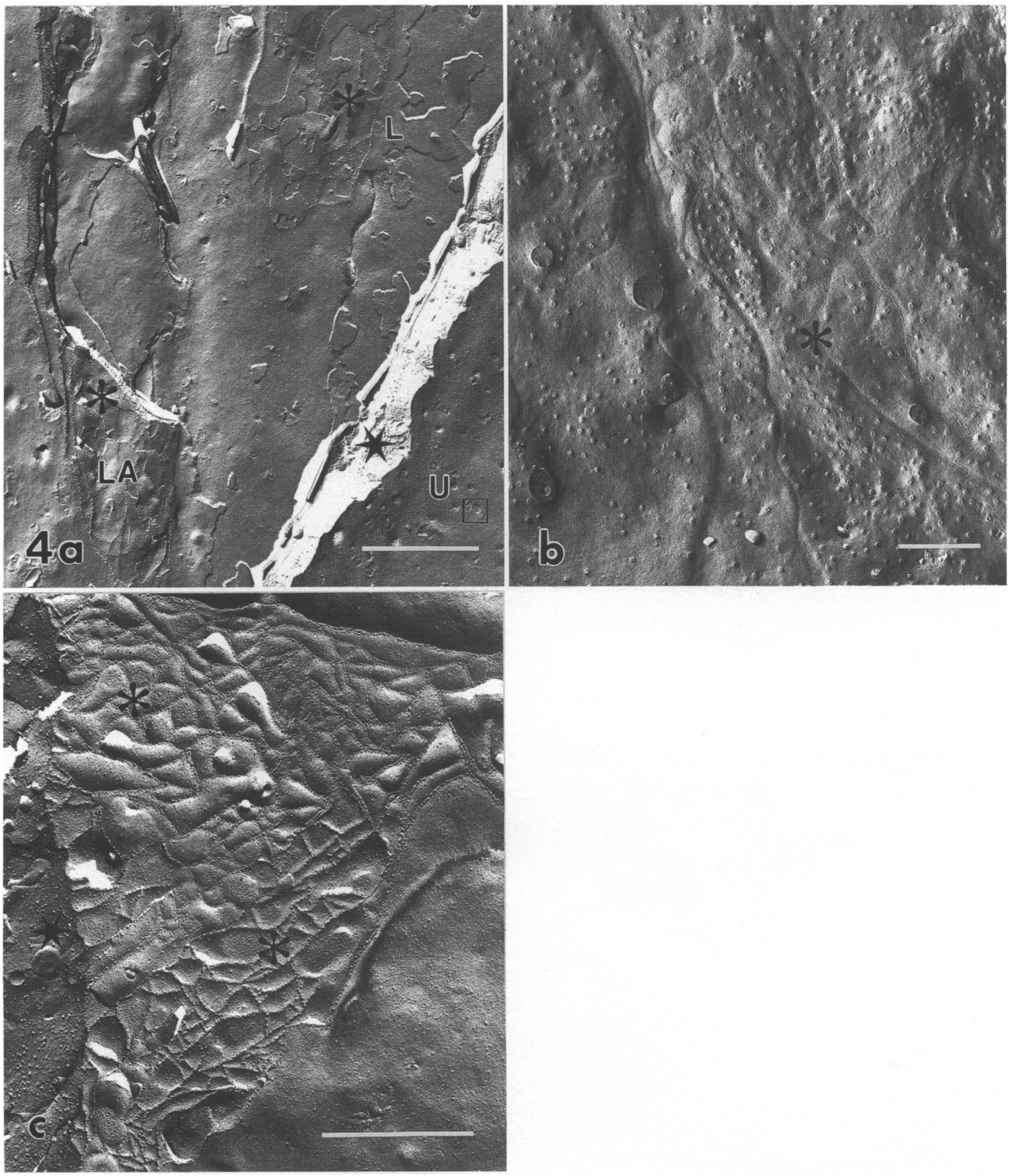

Fig. 4. Freeze-fracture replicas of brain meninges from fish maintained in the dark for 72 h. (*a*) Intermediate layer meningocyte. U, upper membrane; L, lower membrane; LA, lateral membrane. Note the presence of junctional complexes (asterisks) and cross-fractured cells (star). Bar, 1 μm. (*b*) E face of the lower membrane of an intermediate layer meningocyte (note the abundance of plasmalemmal vesicles). Junctional complexes are indicated by an asterisk. Bar, 1 μm. (*c*) Lateral area of an intermediate layer meningocyte. Note the absence of gap junctions. The asterisks label desmosomes and the star the cell cytoplasm. Bar, 1 μm.

showed gap junctions with a rounded or elliptical shape (asterisks in Fig. 2*a*) the size of which was similar to that of the control group (0.06–0.20 μm²). In the lower membranes the number of tight junction strands was less than in the control group and, although in some instances there appeared to be an association between gap and tight junctions (see small arrow in Fig. 2*b*), frequently the 2 types of junction appeared to be separated (large arrows in Fig. 2*b*); in addition, the number of desmosomes was decreased.

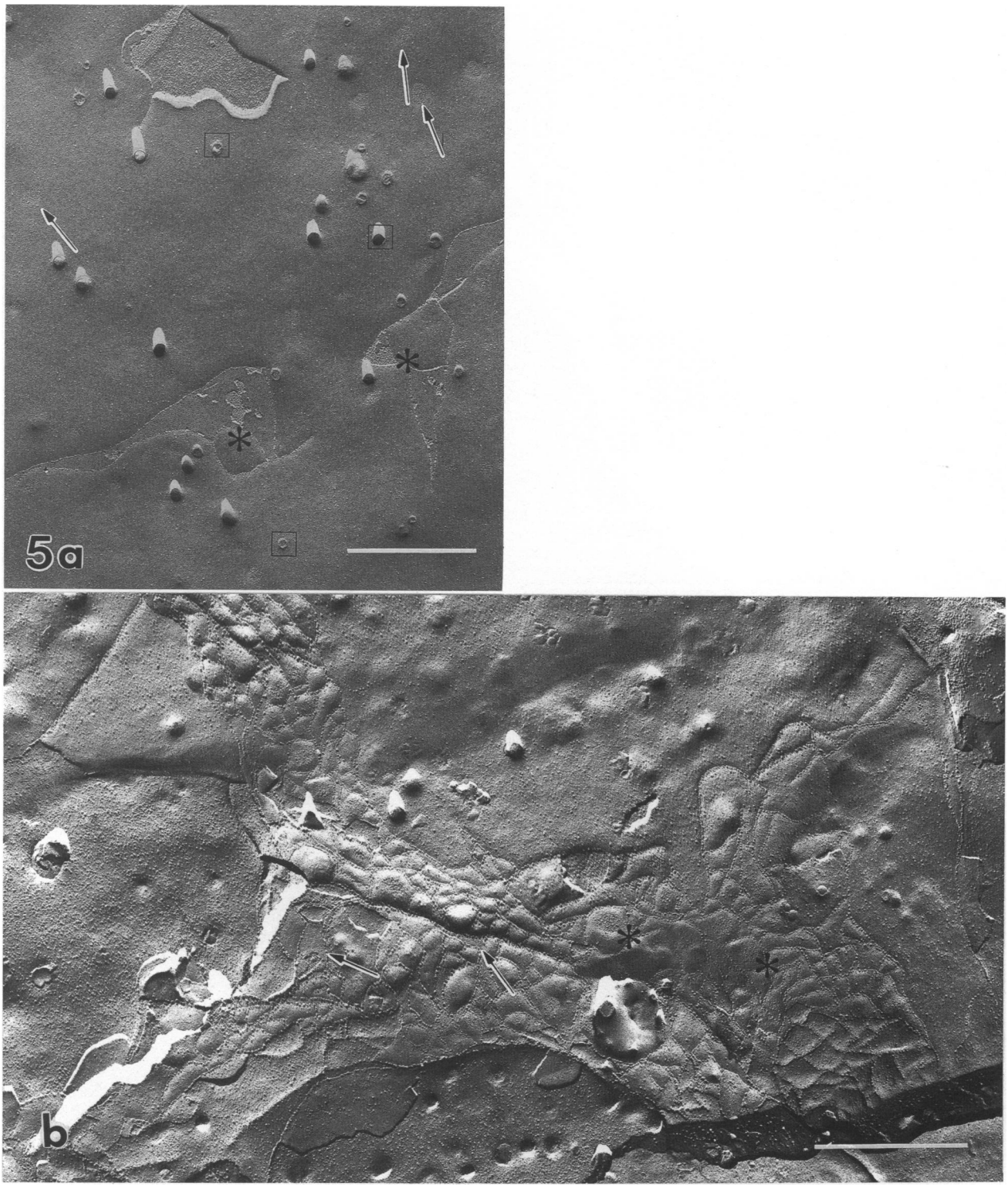

Fig. 5. Freeze-fracture replicas of brain meninges from fish maintained in the dark for 96 h. (*a*) E face of the lower membrane of an intermediate layer meningocyte. Note the presence of gap junctions (asterisks), tight junctions and small desmosomes (arrows); squares indicate plasmalemmal vesicles. Bar, 1 μm. (*b*) Lateral area of an intermediate layer meningocyte. Note the presence of tight junctions, desmosomes (asterisks) and small gap junctions (arrows). Bar, 1 μm.

The morphology of the junctions in the lateral area was similar to that of the control group.

In the group of fish maintained in the dark for 48 h the number of large size gap junctions ($> 0.20\ \mu m^2$) in the upper membrane of the meningocytes was increased and gap junctions therefore represented a greater proportion of the upper plasma membrane surface. Whereas in meninges from other groups gap junctions occupied ~ 3–5% of the total cell surface, in meningocytes of animals from this group, gap

junctions occupied ~ 17–20% of the membrane surface (see Fig. 3*a*). In the lower plasma membrane (Fig. 3*b*) and in the lateral areas, the morphology of the junctions was similar to that in the control group.

Replicas from the group maintained in darkness for 72 h showed intercellular junctions similar to those of the control group, in the upper as well as in the lower membranes (Fig. 4*a*, *b*). However, there were some changes in the junctional complexes of the lateral areas, as is observed in Figure 4*c*. The junctions consisted of a hexagonal network of tight junctions with desmosomes located inside the hexagons. No gap junctions were seen in association with the tight junctions.

Cells from fish maintained in the dark for 96 h showed junctional complexes that were similar to those of the control group. A view of the lower plasma membrane of a meningocyte from this group is shown in Figure 5*a*. Where gap junctions were associated with tight junctions, small desmosomes without connection with other junctional types were also abundant (see arrows in Fig. 5*a*). The lateral area showed tight junctions, desmosomes, and also gap junctions in association with tight junctions (see arrows in Fig. 5*c*).

DISCUSSION

Our experiments have shown important morphological modifications in the plasma membrane of teleost intermediate layer meningocytes during adaptation to darkness. (1) There are no significant changes in the density of nuclear pores of these cells. Nuclear pore density has been shown to be correlated with the degree of transcriptional activity (Benavente et al. 1989; Goldfarb, 1989). The absence of changes in the density of nuclear pores in the intermediate layer meningocytes therefore appears to indicate that the changes in the number of plasmalemmal vesicles and in the morphology of intercellular junctions are not correlated with a significant change in the quantity of transcriptional activity by these cells. (2) We have also shown an increase in the number of plasmalemmal vesicles in the upper and lower membranes of the meningocytes; this increase is maximal in the group maintained for 72 h in the dark, while in the group with 96 h dark adaptation the density of plasmalemmal vesicles decreases to a basal value (similar to that of the control group). The density of plasmalemmal vesicles estimated may be higher than that in the tissue in vivo because it has been shown that glutaraldehyde fixation increases artifactually the number of plasmalemmal vesicles in comparison with quick freezing (Frokjaer-Jensen, 1991; Wagner & Andrews, 1991). However, it is unlikely that the relative proportions observed in different experiments are altered (Severs, 1988). In endothelial cells the plasmalemmal vesicles could participate in the trans-endothelial transport of some molecules (transcytosis) (Palade & Bruns, 1968; Milici et al. 1987; Heltianu et al. 1989) or, as other reports suggest, the system of endothelial vesicles could form functional channels sporadically (Clough & Michel, 1981; Frokjaer-Jensen et al. 1988; Frokjaer-Jensen, 1991). In any case, it appears that an increase in the density of plasmalemmal vesicles (as seen in our experiments) could indicate an increase in the rate of transport of molecules across the intermediate layer cells. (3) There is also an increase of the surface occupied by gap junctions in the upper membranes of the meningocytes from the group of 48 h in darkness. We suggest that this increase may imply a higher level of communication between these cells and the cells in the outer endomeningeal layer that are located directly over the cells of the intermediate layer (Momose et al. 1988; Caruncho et al. 1993).

It is possible that during the process of adaptation to darkness there could be a depletion of the brain ependymins as occurs in other adaptative and training conditions (Benowitz & Shashoua, 1977; Shashoua, 1979; Pironi & Schmidt, 1988; Schmidt & Shashoua, 1988). This depletion in the level of ependymins is restored by the de novo synthesis (Shashoua, 1982, 1985) that takes place in the meninges (Konigstorfer et al. 1990; Hoffmann, 1992; Schmidt et al. 1992). We therefore speculate that an activation in the production of ependymins by meningeal cells could be associated with some of the morphological changes observed during adaptation to darkness. The new ependymins produced in the meninges may reach other parts of the brain by an unknown transport mechanism (see Konigstrofer et al. 1990). It is plausible that in part the passage of ependymins across the intermediate layer is aided by the increase in density of plasmalemmal vesicles in the cells of the intermediate layer in the groups kept for 48 and 72 h in the dark. When the level of ependymins returned to basal levels the changes in cell morphology also reverted to normal. This could correspond to the morphology of the intermediate meningocytes in the group maintained for 96 h in the dark, which is similar to that of meningocytes of fish from the control group. The ependymins might be produced by cells of the outer or inner leptomeningeal layers, although perhaps by cells of the inner layer because of their particular morphological characteristics (Momose

et al. 1988; Caruncho et al. 1993). We are currently studying possible anatomical changes in the inner layer meningocytes after changes in the photoperiod.

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