Responses of the PAS-positive pars intermedia cells in the cichlid fish *Sarotherodon mossambicus* to ambient calcium and background adaptation

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Summary. The function of the PAS-positive pars intermedia cells in fish has been connected with control of background adaptation and of plasma calcium levels. Since background reflectivity and illumination influence calcium metabolism in *S. mossambicus*, we have tested the hypothesis that the effects of background reflectivity on the PAS-positive cells are mediated by changes in plasma calcium. However, total plasma calcium does not correlate with the activity of the PAS-positive cells as estimated by morphometrical criteria. Transfer of *S. mossambicus* to a white background leads to a drop in plasma calcium, and a marked reduction of the activity of the PAS-positive cells. Adaptation to low-calcium freshwater, on a neutral background, has the same effect on plasma calcium but has no effect on the PAS-positive cells. The characteristic structural features of highly active PAS-positive cells in fish from a black background are not due to the slight hypocalcemia that occurs in these fish, since addition of calcium to the water in concentrations that fully prevent the drop in plasma calcium does not suppress the PAS-positive cells. These findings make it very unlikely that these cells produce a hypercalcemic factor in *S. mossambicus*.

Key words: Pituitary – Pars intermedia – PAS-positive cells – Calcium regulation – Teleost fish

As in other teleosts, the pars intermedia in *Sarotherodon mossambicus* (*Tilapia mossambica*) contains two histologically distinct cell types. The predominant cells produce MSH and a variety of other melanotropic and non-melanotropic peptides (Van Eys 1981; Van Eys and Van den Oetelaar 1981), and are thus similar to the MSH cells of the pituitary gland in higher vertebrates. In *S. mossambicus*, the MSH cells are involved in the endocrine control of integumental pigmentation (Van Eys 1980a; Van Eys and Peters 1981).

The function of the other cell type, termed PAS-positive cells or PIPAS cells because of their affinity for the periodic acid-Schiff histological stain, and found in many teleost species, has been connected with the control of processes as diverse as background adaptation and ion regulation. In several species (Baker 1963; Baker and Ball 1970; Malomichele 1977), including *S. mossambicus* (Van Eys 1980b), the PAS-positive cells are activated when fish are kept on a black background. Therefore, a role for these cells in the control of skin pigmentation has been proposed. Some investigators have reported stimulation of the PAS-positive cells in response to variations in environmental salinity (Olivereau 1967, 1969; Leatherland 1970; Baker 1972). Recently, however, attention has shifted from salinity in general to calcium in particular.

In eels, goldfish and killifish, the PAS-positive cells are activated by a reduction in external Ca$^{2+}$ concentration (Olivereau et al. 1980, 1981; Olivereau and Olivereau 1982; Ball et al. 1982) and they have been designated as Ca-sensitive cells (Olivereau et al. 1980). The unknown factor produced by the PAS-positive cells has been tentatively considered a hypercalcemic hormone (Olivereau et al. 1980; Ball et al. 1982).

We have suggested a role for the PAS-positive cells of *S. mossambicus* in the control of calcium metabolism, although we have found that the activity of these cells appears to be determined by background reflectivity and illumination (Van Eys 1981). This suggestion is based on (1) the absence of a correlation between skin pigmentation and activity of the PAS-positive cells (Van Eys and Peters 1981), (2) unpublished observations, showing that stimulation of the PAS-positive cells in fish kept in total darkness is accompanied by a severe although transient drop in plasma calcium.

To investigate the possibility that activation of the PAS-positive cells is a response to a drop in plasma calcium levels (as may be expected from cells producing a hypercalcemic hormone) we have studied the PAS-positive cells with respect to the adaptation of fish to different ambient calcium levels and different background reflectivity.

Materials and methods

Sexually mature males of *S. mossambicus* with a body weight between 15 and 20 g were used. The fish were reared in freshwater tanks at 26 °C and a daily light period of 12 h (from 8:00 to 20:00 h). The water contained 2.1 mM Na$^+$, 0.5 mM Cl$^-$, 0.4 mM SO$_4^{2-}$ and 0.8 mM Ca$^{2+}$ ("nor-
Figs. 1, 2. PAS-positive cells of fish adapted to a white background (Fig. 1) or to a neutral background (Fig. 2), both in normal freshwater; Ga Golgi area; ger granular endoplasmic reticulum; sc stellate cells; MSH MSH cells; a axon profiles of the neural part of the pituitary gland. × 10000

mal freshwater”). The fish were fed twice daily with Tetra-min tropical fish food and minced beef heart. Illumination was similar for all experimental tanks used, as described earlier (Van Eys 1980a).

Six groups of fish were studied and kept for a period of 4 weeks under one of the following conditions:

a) White background, normal freshwater. Fish were kept in white containers as described by Van Eys (1980a).

b) Neutral background, normal freshwater. Controls for groups c and d; fish were kept in glass containers with gravel.

c) Neutral background, high-calcium freshwater. As in b, but the Ca²⁺ concentration of the water was increased every other day by adding CaCl₂; a final concentration of 10.2 mM Ca²⁺ was reached by day 6 of the experimental period.

d) Neutral background, low-calcium freshwater. As in b, but the Ca²⁺ concentration of the water was reduced by replacing part of the water every other day with Ca²⁺-free artificial freshwater; a final concentration of 0.02 mM Ca²⁺ was reached by day 6 of the experimental period; the concentrations of the other major ions and the pH were kept at the same level as in normal freshwater; the fish were fed low-calcium food (beef heart).

e) Black background, normal freshwater. Controls for group f – fish were kept in black non-reflective containers as described by Van Eys (1980a).

f) Black background, high-calcium freshwater. Fish kept in black containers (as in e) were adapted to high-calcium water (as in c). The Ca²⁺ concentrations of the water were determined weekly, and adjusted as necessary. At the end of the experimental period, the fish were lightly anesthetized with MS-222, and blood was collected from the severed caudal blood vessels. After decapitation, the pituitary glands were quickly removed and processed for light or electron microscopy. For technical details concerning fixation and embedding, determination of cell and nuclear volumes, morphometrical analysis of electron micrographs, and statistical evaluation, see Van Eys (1980a). Morphometrical analysis was performed on 5 fish per group. Plasma osmolality was determined with a Vogel micro-osmometer, and total plasma calcium by atomic absorption spectrophotometry, in the presence of lanthanum chloride. These blood parameters were determined for 8 fish per group. The experiments were carried out in summer.
Results

Morphometry of the PAS-positive cells

A detailed description of the pars intermedia and its endocrine cell types in S. mossambicus has been published previously (Van Eys 1980a, b).

White background, normal freshwater (group a). In fish adapted to a white background, the PAS-positive cells were small and low in number (Fig. 1). Morphometrical analysis at the light and electron microscopical levels showed that cell and nuclear volumes (P<0.001), and the volume densities of mitochondria (P<0.01) and Golgi areas (P<0.05) were all significantly lower than in the other experimental groups (Fig. 3).

Neutral background (groups b, c and d). In control fish from normal fresh-water (group b), the PAS-positive cells were numerous, large and well developed. Granular endoplasmic reticulum and Golgi areas were extensive and many electron-dense secretory granules were present (Fig. 2). The structure of the PAS-positive cells of fish from water with high (group c) or low (group d) calcium concentration was similar to that of controls from normal freshwater (Figs. 3-5).

No significant differences could be demonstrated in cell or nuclear volumes, or in the volume densities of mitochondria, granular endoplasmic reticulum, and Golgi areas.

Black background (groups e, f). In fish adapted to a black background in normal freshwater (group e) the cell and nuclear volumes of the PAS-positive cells were enlarged compared with controls from a neutral background (group b; P<0.01; Fig. 3). The values for the ultrastructural parameters were similar. No differences could be observed between the PAS-positive cells of the high-calcium group (f) and its control (group e; Figs. 3, 6, 7).

Plasma osmolality and total plasma calcium

Compared with control fish from a neutral background (group b), fish from a white background and from normal freshwater showed reduced plasma osmolality and total plasma calcium (Table 1). This reduction may be due to stress caused by a prolonged stay on a white background. Stress may also be the reason that fish on a white background showed a high mortality when adapted to low calcium freshwater (unpublished observation).

In fish from a neutral background (groups b, c, d), exposure to water with a high calcium concentration (group c) resulted in a slight but non-significant increase of total plasma calcium. Fish from low-calcium water showed a slight but significant decrease in this value (group d). Groups d and c differed significantly for this parameter. Plasma osmolality was reduced under both high- and low-calcium conditions when compared with that of fish from normal freshwater. In fish from a black background, high-calcium water (group f) did not influence plasma osmolality but it increased total plasma calcium significantly in comparison with that of fish from normal freshwater (group e).

Discussion

The present morphometrical findings confirm earlier observations on S. mossambicus (Van Eys 1980b) that the PAS-
positive cells are inactive in fish adapted to a white background, and stimulated in fish on a black background. The data on total plasma calcium show that background reflectivity has effects on calcium metabolism. However, exposure of fish to both white and black background results in a reduction of plasma calcium levels. This makes untenable the hypothesis that the effects of background reflectivity on the PAS-positive cells are mediated by changes in plasma calcium. This conclusion is supported by other observations: 1) exposure of fish to low-calcium water produces the same decrease in plasma calcium as exposure to a white background but does not affect the activity of the PAS-positive cells; 2) exposure of fish to high-calcium freshwater significantly enhances plasma calcium when compared to that of the low-calcium group, but does not influence the PAS-positive cells; 3) the high activity of the PAS-positive cells of fish from a black background is not notably modified by an increase in external calcium that results in mild hypercalcemia. The PAS-positive cells of *S. mossambicus* are therefore not likely to produce a hypercalcemic factor, as has been suggested for some other teleost species (Olivereau et al. 1980; Ball et al. 1982).

The values for plasma osmolality reported in this paper cannot be related to the activity of the PAS-positive cells. Our unpublished data on *S. mossambicus* have further shown that transfer of fish from freshwater to seawater does not evoke any response in the PAS-positive cells. Thus, neither external nor internal osmolality appear to influence these cells.

The above results on the effects of external calcium and osmolality on the PAS-positive cells in *S. mossambicus* are in line with findings reported for species such as *Poecilia latipinna* (Ball and Batten 1981) but contrast with data reported for eel, goldfish (Olivereau et al. 1980; Olivereau and Olivereau 1982), and killifish (Ball et al. 1982). Similarly, the responses to background of the PAS-positive cells (cf. those of the MSH cells) differ widely in teleosts. On the basis of the background adaptation response of the pars intermedia endocrine cells, Ball and Batten (1981) distinguish three groups of teleosts: group 1, including Anguilla, Phoxinus and Salmo in which adaptation to a black background activates the MSH-cells; group 2, including Blennius, Cichlasoma and Poecilia, in which the PAS-positive cells are activated; and group 3, including Sarotherodon, Boops and Scorpaena, in which both the MSH and PAS-positive cells are activated on a black background. It has been reported that in *Anguilla anguilla* (group 1) the PAS-positive cells respond to changes in salinity and calcium content of the water, whereas the MSH cells show minor changes (Olivereau et al. 1980; Olivereau and Olivereau 1982). In *Poecilia latipinna* (group 2) no effects of salinity changes or transfer to calcium-free seawater could be observed (Ball and Batten 1981). Similarly, our present and unpublished data show no differences in the PAS-positive
Figs. 6, 7. PAS-positive cells from fish adapted to a black background in water with normal calcium (Fig. 6: 0.8 mM Ca\(^{2+}\)) or high calcium (Fig. 7: 10.2 mM Ca\(^{2+}\)); Ga Golgi area; ger granular endoplasmic reticulum; MSH MSH cells. ×10000
and MSH cells of *S. mossambicus* (group 3) in response to changes in ambient calcium or salinity. The latter result is in line with the inference of Ball and Batten (1981) that major responses to background reflectivity and to salinity or calcium are alternative reactions of the PAS-positive cells, a feature which may be incompatible within any one species.

It should be kept in mind, however, that there is at present no conclusive experimental evidence from any species that the PAS-positive cells are implicated in the endocrine control of either background adaptation or of calcium metabolism and osmoregulation. Since the purified products of the PAS-positive cells are not available for experimentation, only correlations between PAS-positive cell activity (estimated on the basis of cell morphology) and environmental stimuli can be demonstrated. In *S. mossambicus*, but PAS-positive and MSH cells are activated on a black background and inactivated on a white background. Adaptation to black background is accompanied by darkening of the skin and increased melanogenesis, whereas adaptation to white background results in paling of the skin, due to reduction in size and number of the melanophores (Van Eys and Peters 1981). However, after transfer to complete darkness, when skin colour is intermediate between that exhibited on black and white background, the secretory activity of the MSH cells is also intermediate, whereas that of the PAS-positive cells is very high (Van Eys 1980b).

In addition, we have demonstrated that melanogenesis and number of melanophores in the skin are regulated by aMSH (Van Eys 1980a). However, after transfer to complete darkness, when skin colour is intermediate between that exhibited on black and white background, the secretory activity of the MSH cells is also intermediate, whereas that of the PAS-positive cells is very high (Van Eys 1980b).

### Table 1. Plasma osmolality and total calcium concentrations in *S. mossambicus* kept on a white, neutral or black background for 4 weeks in water with different Ca$^{2+}$ levels; means ± S.D. of 8 fish per group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Background</th>
<th>Water Ca$^{2+}$ concentration (mM)</th>
<th>Plasma osmolality (mosmol/l)</th>
<th>Plasma calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>white</td>
<td>0.8</td>
<td>316.0 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.66 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>b</td>
<td>neutral</td>
<td>0.8</td>
<td>332.1 ± 2.4</td>
<td>2.94 ± 0.17</td>
</tr>
<tr>
<td>c</td>
<td>neutral</td>
<td>10.2</td>
<td>323.4 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>d</td>
<td>neutral</td>
<td>0.02</td>
<td>320.2 ± 3.6</td>
<td>2.69 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>e</td>
<td>black</td>
<td>0.8</td>
<td>328.7 ± 4.1</td>
<td>2.73 ± 0.18</td>
</tr>
<tr>
<td>f</td>
<td>black</td>
<td>10.2</td>
<td>323.0 ± 6.7</td>
<td>3.19 ± 0.22&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> significantly different from b 
<sup>b</sup> significantly different from d 
<sup>c</sup> significantly different from b 
<sup>d</sup> significantly different from e 

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