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Effect of Methallibure on the Hypophysis and Gonadal Development of the Cichlid Fish, *Tilapia mossambica**

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Abstract The present paper describes histological and cytological changes in the hypophysis and gonads of juvenile *Tilapia* (=*Sarotherodon*) *mossambica* exposed to the antigonadotropic drug methallibure (I.C.I. 33,828) by means of daily doses of 1 or 2 p.p.m. for 7 weeks. This treatment markedly prevented the process of maturation of gametogenesis in both sexes of fish, in addition to retardation of differentiation of interstitial cells and epithelial lining of the efferent duct of the testis. The polygonal basophils (gonadotrophs) located in the ventral portion of the proximal pars distalis of the hypophysis underwent a marked decrease in the cell size as well as in the amount of cytoplasmic granules of glycoproteinaceous nature. Atrophy was manifested by the acidophils (somatotrophs) of the proximal pars distalis and the PAS positive cells of the pars intermedia, whereas no appreciable change was recognized in other cell types of the adenohypophysis.

Hoar et al. (1967) first demonstrated the antigonadotropic effect of dithiocarbamoylhydrazine derivative methallibure (I.C.I. 33,828) in three species of teleost fishes. Since then, many investigators have tried to apply this drug as a useful means for analysis of the hypophyseal-gonadal axis of these animals, because hypophysectomy is not always feasible in teleost species (Wiebe, 1968; Martin and Bromage, 1970; Pandey and Leatherland, 1970; Mackay, 1973a, b; Van den Hurk and Van de Kant, 1975; Van den Hurk and Testerink, 1975; Singh et al., 1977). In *Tilapia* species as well as in other teleost fishes, methallibure treatment causes a pronounced regression of both gonads, such as retardation of spermatogenesis and vitellogenesis, atrophy of androgen producing cells (Hyder, 1972; Hyder et al., 1974) as well as certain change in skin pigments (Lanzing, 1978). However, no data are available on the effects of methallibure on the hypophysis of this fish.

In the present study, therefore, the hypophysis of methallibure-treated *Tilapia* (= *Sarotherodon*) *mossambica* were examined to evaluate the drug-induced cytological changes, which in turn lowers the carotenoid concentration of the dorsal fin (Lanzing, 1978).

Material and Methods

Fifteen juvenile fish of both sexes of *Tilapia mossambica*, recently renamed *Sarotherodon mossambicus*, about 12 weeks old, were used in this study. They were divided into 3 groups consisting of 5 fish each and placed separately in small aquaria, each of which contains 10 liter water at a temperature of 24°C. Two groups received daily doses of 1 and 2 p.p.m. of methallibure (I.C.I. 33,828) for 7 weeks, respectively. The control group only received a daily dose of the dispersant Tween. Details of the experimental procedure are described elsewhere (Lanzing, 1978). After decapitation, brain with hypophysis as well as gonads were removed and fixed in Bouin-Hollande solution. These materials were dehydrated, embedded in paraffin, cut serially 7 μ thick in sagittal direction, and stained with various stainings such as azan trichrome, aldehyde fuchsin (AF)-fast green-orange G, periodic acid Schiff (PAS)-fast green-orange G, lead hematoxylin (PbH) and Delafield's hematoxylin-eosin. To estimate the activity of the glandular cells in the hypophysis, cell index, (maximum cell length

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+maximum cell width)/2, and mean nucleus diameter, (longer axis+shorter axis)/2, of 10 cells of each cell type examined were measured. In addition, the number of cell nuclei in a defined area in 20 different regions containing only prolactin cells or gonadotrophs were counted to evaluate the changes in cell size.

Results

General structure of the hypophysis in the control fish

The general structure and developmental processes of the hypophysis of *Tilapia mossambica* have already been reported by Sasayama and Takahashi (1975) and the present examination confirmed their description. The hypophysis of the juvenile fish possesses a more depressed form than that of the adult, mature fish, but four regions were well-differentiated: the rostral pars distalis (RPD), the proximal pars distalis (PPD), the pars intermedia (PI), and the pars nervosa (PN) (Fig. 1).

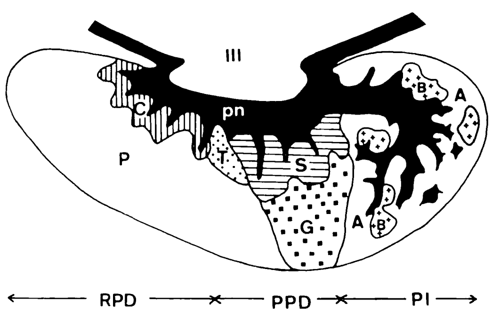

Fig. 1. Diagrammatic representation of the hypophysis of the juvenile cichild, *Tilapia mossambica*. Anterior to the left. III (third ventricle), pn (pars nervosa), RPD (rostral pars distalis), PPD (proximal pars distalis), PI (pars intermedia), C (corticotrophs), P (prolactin cells), T (thyrotrophs), S (somatotrophs), G (gonadotrophs), A (PbH positive cells), B (PAS positive cells).

Rostral pars distalis. The RPD covering the antero-ventral region of the hypophysis contains two types of granulated cells. In the most dorsal part bordering the neurohypophyseal processes small cells, columnar or fusiform in shape, were found. They gathered to form single or two-cell layered cell cords. These cells were stained bluish gray with PbH and pale pink with azan trichrome. The nucleus, oval or round in shape, had an indistinct nucleolus. Judging from the topographical distribution and staining properties these cells should be regarded as the corticotrophs. The rest of the RPD was occupied exclusively with typical eta (acidophil) cells, which are known as the prolactin cells. They were polygonal or round in shape, having a nucleus with one or two prominent nucleoli. The well developed cytoplasm showed a coarse granular condition and was stained reddish orange with azan stain. In addition, agranular cells characterized by a scanty cytoplasm including a small nucleus with an irregular wavy margin were frequently found interposed among the prolactin cells and/or between the capillary walls and prolactin cells. These cells may correspond to so-called stellate cells.

Proximal pars distalis. Three types of cells could be distinguished in the PPD. In the antero-dorsal region of the PPD small basophils corresponding to the thyrotrophs were grouped in small clusters. This cell type is angular or polygonal in shape and has a small nucleus with an indistinct nucleolus. The cytoplasma is scanty and contains fine granules showing a weak affinity for AF, PAS and aniline blue. The acidophils diagnosed as somatotrophs were situated in the most dorsal region of the PPD to form single or two-cell layered cell cords that border the neurohypophysis (Figs. 1, 4). A few of them, however, were occasionally encountered among the basophils located ventrally. This acidophil containing an ovoid nucleus may show up in various shapes such as columnar, polygonal and ovoid. The size and staining affinity of the cells tended to vary from cell to cell. The polygonal basophils identical with gonadotrophs were stained deeply with AF, PAS and aniline blue and predominated in the ventral portion of the PPD (Fig. 2). They were characterized by the presence of two kinds of glycoproteinaceous granules in the cytoplasm: one had fine, cyanophilic granules and the other orangenophilic globules by azan trichrome. Regarding number, de-

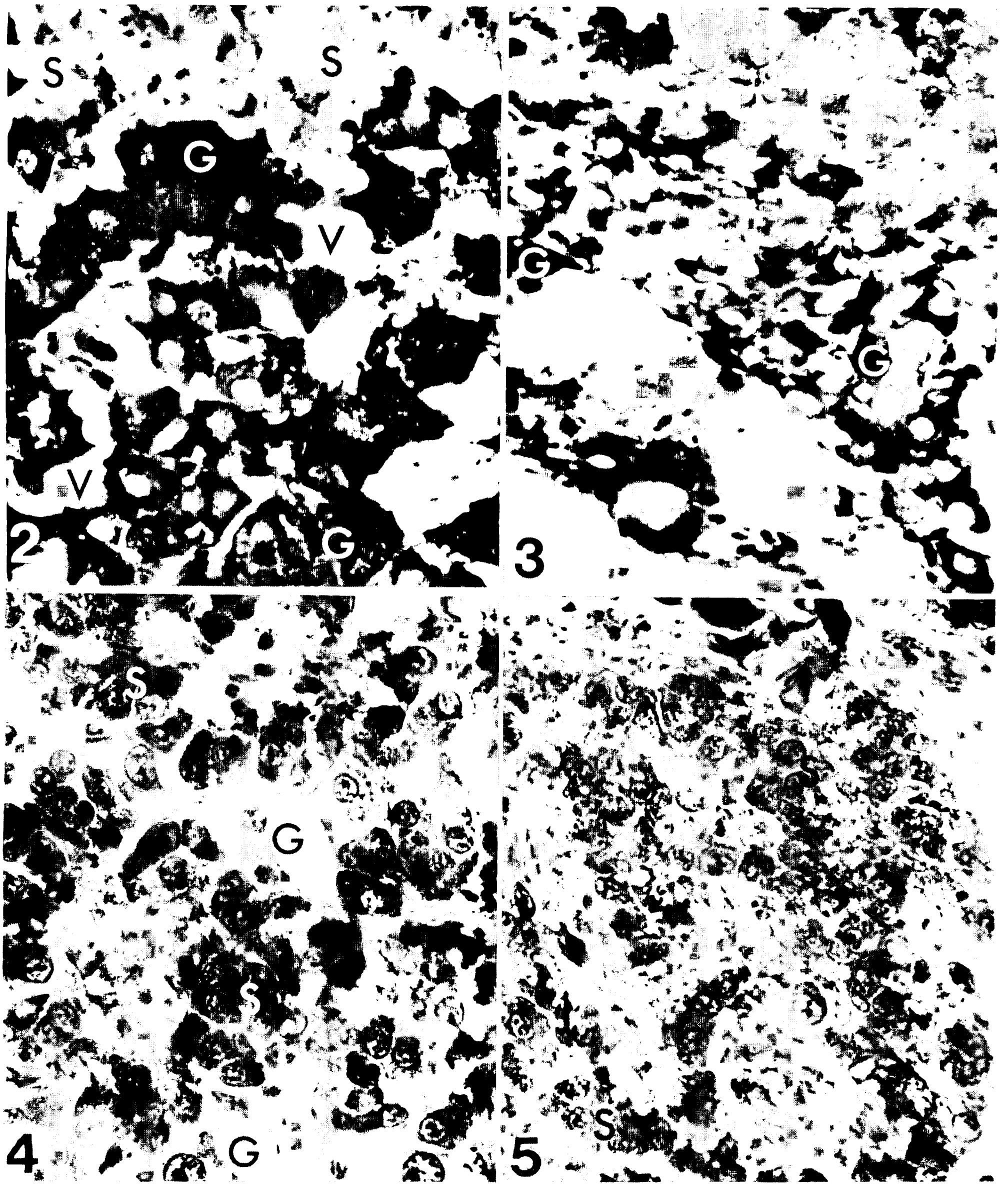

Fig. 2. Part of the PPD of the control fish showing heavily granulated gonadotrophs (dark cells, G) and lightly stained somatotrophs (S). Note the large vacuoles (V) in the gonadotrophs. AF-fast green-organge G stain, 1000×. Fig. 3. Part of the fish treated with methallibure (1 p.p.m./day for 7 weeks). Note a marked atrophy occurring in the gonadotrophs (G) with poorly granulated cytoplasm. AF-fast green-orange G stain, 1000×. Fig. 4. Section of the PPD of the control fish showing well-granulated somatotrophs (S) and large gonadotrophs (G) with vacuolized cytoplasm. Azan stain, 1000×. Fig. 5. Section of the PPD of the fish treated with methallibure (2 p.p.m./day for 7 weeks). A heavy atrophy is seen in the somatotrophs (S), which are arranged in cell cords facing the pars nervosa (N). Azan stain, 1000×.

gree of hypertrophy and severity of degranulation of the gonadotrophs, the cells of male fish were more numerous, respectively more affected than those of female fish. This tendency was more pronounced in the dominant male than in the submissive males.

Fig. 6. Detail of the control fish showing two types of cells: darkly stained PbH positive cells (A) and lightly stained PbH negative (PAS positive) cells (B). PbH stain, 1000×. Fig. 7. The detail of the PI of the fish treated with methallibure (1 p.p.m./day for 7 weeks). The atrophic change is seen in the PbH negative (PAS Positive) cells (B). PbH stain, 1000×. Fig. 8. Part

Pars intermedia. The PI was invaded extensively with the neural processes of the PN, but also included two types of cells: the first type was the PbH positive but PAS negative cell, polymorphic in shape. The second type was a PAS positive but PbH negative cell, cuboid or ovoid in shape (Figs. 1, 6). Using azan stain these two types of cells were differentiated as weakly carminophil and orangenophil cells, respectively. The PbH positive cell with coarse granular cytoplasm occasionally came in contact with the neural tissue by its slender, cytoplasmic process. The PAS positive cell was arranged in small cell cords or clusters facing the PN and often appeared scattered singly amongst the PbH cells. The cell had a round nucleus with one or two prominent nucleoli, and its cytoplasm was finely granulated.

Effect of methallibure on the hypophysis

Judging from cytometrical data and staining affinity for dyes, three types of cells were affected by methallibure: gonadotrophs, somatotrophs and the PAS-positive cells of the PI (Table 1).

The gonadotrophs showed a marked atrophy accompanying a decrease or depletion of the glycoproteinaceous granules in the cytoplasm (Figs. 2, 3). The vacuoles and acidophil globules demonstrable in the gonadotrophs of the control fish were no longer detectable in the treated fish. Table 1 shows that the cell index decreased from 9.38 for the control fish to 7.58 and 7.14 for the treated fish (the differences are significant at $p<0.05$). However, the apparent difference in mean nucleus diameter was not significant statistically.

The cell index for the somatotrophs de-

Table 1. Effect of methallibure on cell types in the adenohypophysis of *Tilapia mossambica*.

		Prolactin	Corti-cotroph	Thyrotroph	Somato-troph	Gonado-troph	PbH positive cell	PAS positive cell
Cell index (μ)	control	8.43±0.09[a]	6.30±0.13	5.59±0.18	6.69±0.15	9.38±0.37	8.53±0.08	9.05±0.32
	MTH_1[b]	8.35±0.20	6.39±0.11	5.38±0.17	*5.90±0.14	*7.58±0.12	9.08±0.27	*7.45±0.12
	MTH_2[c]	8.58±0.13	6.05±0.14	5.32±0.05	*5.81±0.08	*7.14±0.30	8.85±0.23	*7.56±0.11
Mean nucleus diameter (μ)	control	3.83±0.03	3.68±0.05	3.28±0.10	3.47±0.04	4.31±0.20	4.18±0.05	3.81±0.06
	MTH_1	3.78±0.03	3.77±0.05	3.30±0.07	3.51±0.03	3.96±0.02	4.24±0.03	3.71±0.02
	MTH_2	3.84±0.04	3.68±0.02	3.23±0.08	3.33±0.03	3.76±0.09	4.07±0.07	3.63±0.05
Number of cell nuclei on a defined area	control	4.8 ±0.1				3.3 ±0.3		
	MTH_1	4.9 ±0.1				**4.3 ±0.2		
	MTH_2	5.0 ±0.1				**5.3 ±0.3		

a) mean±SE
b) methallibure (1 p.p.m./day for 7 weeks)
c) methallibure (2 p.p.m./day for 7 weeks)
* means are significantly different from control ($p<0.05$)
** means are significantly different from control ($p<0.02$)

of the testis of the control fish showing active spermatogenesis and well-developed interstitial tissue (arrow). A mass of spermatozoa is stored in the lumen of the efferent duct lined with the columnar epithelial cells. H-E stain, 350×. Fig. 9. Section of the testis of the methallibure-treated fish (1 p.p.m./day for 7 weeks), showing the predominating spermatogonia (SG) and picnotic nuclei of the spermatocytes (arrow). Note a severe shrinkage occurring in interstitial cells (I) and flattened epithelial cells of the efferent duct (E). H-E stain, 400×. Fig. 10. Part of the ovary of the control fish showing a maturing ovum heavily laden with the yolk globules. Note the well-developed follicle cells surrounding an ovum. H-E stain, 200×. Fig. 11. Part of the immature ovary after methallibure administration (1 p.p.m./day for 7 weeks). The oocytes remains in the yolkless stage. H-E stain, 200×.

creased from 6.69 for the control fish to 5.90 and 5.81 for the treated fish (the difference with the controls are significant at $p<0.05$). The affected somatotrophs showed a weak response affinity for acid dyes (Figs. 4, 5). However, there was no significant difference in mean nucleus diameter of the somatotrophs between control and treated fish.

A statistically significant ($p<0.05$) decrease in cell index data was observed between the somatotrophs (Figs. 6, 7) of the control fish and those of the treated fish, although the mean nucleus diameter did not change.

No noticeable difference between control and treated fish was detected in the amount of stainable neurosecretory material in the PN.

Effects of methallibure on the gonads

Methallibure markedly prevented the gonadal development in both sexes. The testes of the control fish were characterized by well developed seminiferous tubules showing active spermatogenesis. Also present are well-developed interstitial cells, 11.5μ in mean longer axis, lying in the intertubular spaces. The efferent duct is linked with a tall epithelium consisting of secretory cells, 12.4μ in mean height (Fig. 8). The tubule was occupied mainly by spermatocytes and spermatids, although a few spermatogonia still existed. Moderate amounts of sperm and secretory product were stored in the lumen of the efferent duct. The interstitial cell had a round nucleus with one or two prominent nucleoli. Its cytoplasm was slightly foamy and stained with eosin. On the other hand, the inhibition of the testicular development in the methallibure-treated fish was remarkable, indicating an immature condition characterized by the predominance of spermatogonia. Spermatocytes and spermatozoa were rarely observed (Fig. 9), but some of the spermatocytes showed picnotic nuclei. A regressive change was also evident in the interstitial tissue and epithelial linings of the efferent duct after administration of methallibure (Fig. 9). A decrease in size and number of the interstitial cells was evident and accompanied by a shrinkage of nuclei. The size of the cells was reduced by about a half when compared to the controls (5.5$\sim$5.8μ in diameter). The epithelial cells of the efferent duct became markedly flattened, (4.1 to 4.2μ in height) suggesting a hypofunctional state.

The control females possessed ovaries crowded with fully grown ova measuring about 1,200μ in diameter. The ooplasm was heavily laden with yolk globules of various sizes (Fig. 10). A single layer of the follicle epithelium enclosing the mature oocytes consisted of cuboid cells. The cell was characterized by a round nucleus and granular cytoplasm staining with eosin (Fig. 10). Regarding methallibure-treated fish, the vitellogenesis and differentiation of the follicle cells were completely blocked, that is, the ovaries retained an immature state and their ovigerous folds were filled with numerous young oocytes, less than 200μ in diameter. In addition, a few cysts of oogonia were scattered amongst them (Fig. 11). Otherwise, no apparent abnormalities were found among these young oocytes and oogonia.

Discussion

The present study demonstrates that there are seven types of granular cells in the adenohypophysis of juvenile *Tilapia mossambica*. The cytological characteristics of these seven cell types are closely similar to the descriptions of Leatherland et al. (1974) in *Tilapia* spp. and Sasayama and Takahashi (1975) who used the same species as in the present examination.

Confirming previous reports by several investigators on different teleosts (Leatherland, 1969; Billard et al., 1970; Pandey and Leatherland, 1970; Mackay, 1971; Van den Hurk and Van den Kant, 1975; Van den Hurk and Testerink, 1975), the present investigation revealed that methallibure effectively inhibits the differentiation of the gonadotrophs of *Tilapia* hypophysis and causes the prevention of spermatogenesis and vitellogenesis. Regression of the interstitial tissue and epithelial linings of the efferent duct in the testis and suppression of the growth of the ovarian follicle cells are also brought about. These findings support the view that methallibure blocks synthesis and/or release of the pituitary gonadotropin. Thus, the antigonadotropic effect of methallibure documented in previous studies on *Tilapia* (Hyder, 1972; Hyder et al., 1974; Lanzing, 1978) is confirmed by the present examination. Recently, Singh et al. (1977) presented an interesting result, indicating that methallibure altered the effect of exogenous gonadotropins (LH and/or FSH) on the catfish ovary. These observations partially contrast with the results obtained by Wiebe (1968, in *Cymatogaster*) and Hyder (1972, in *Tilapia*). According to these investigators, the inhibitory effect of methallibure on testicular development and spermatogenic activity was reversed after treatment with peripherally administered LH or *Tilapia* pituitary homogenate, despite continued treatment with methallibure. Consequently, it appears that the influence of methallibure on the effects of exogenous gonadotropin on the gonads requires further study. Whether methallibure acts directly on the gonadotrophs or indirectly via hypothalamus is uncertain. However, Kitay and Westfall (1977) suggested that methallibure may exert its primary action at the hypothalamic sites involving a catecholaminergic pathway, subsequently modifying the hypothalamic regulation of the endocrine activity of the hypophysis.

An atrophic change with concomitant degranulation was shown by the somatotrophs of *Tilapia* administered with methallibure. The same has already been reported in *Cymatogaster* (Leatherland, 1969) and *Poecilia* (Pandey and Leatherland, 1970). However, a possible relation between this change and the growth rate of the present fish was not ascertained. It is difficult to explain the significance of the inhibitory effect of methallibure on the somatotrophs and on the PAS positive cells of as yet unknown function. The antithyroid effect of this drug found in mammals (Walpole, 1965) has also been demonstrated in some teleostean species as evidenced by hyperactive thyroid follicle and hypertrophied thyrotrophs (Leatherland, 1969; Pandey and Leatherland, 1970; Mackay, 1971). However, the grade of this effect was less than that of thiourea (Pandey and Leatherland, 1970; Mackay, 1975a), while there is no effect in the thyroid of the adult goldfish (Hoar et al., 1976) and the juvenile guppy (Pandey and Leatherland, 1970). In the present case of *Tilapia*, no appreciable change was observed in the thyrotrophs by means of light microscopy. Accordingly, the present results confirm only that methallibure effectively inhibits the gonadotropic activity of the hypophysis, with a concomitant suppression on the gonadal development.

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ティラピアの下垂体ならびに生殖腺の発達に及ぼすメサリビュアの影響

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ティラピア幼魚の下垂体ならびに生殖腺の発達に対する生殖腺刺激作用阻害剤（メサリビュア）の影響を知るために，細胞学的および組織学的に観察を進めた．実験群は，7週間にわたり毎日 1 ppm 投与したものと，2 ppm 投与したものとの2群よりなる．本処理により，雌雄共に，配偶子形成の成熟が遅延し，また精巣の間質細胞と導出管上皮の分化が阻害された．一方，下垂体端葉軸部の腹方に存在する生殖腺刺激細胞においては，細胞の大きさならびに糖たん白性顆粒の量の減少が目立った．また，同部にある成長ホルモン分泌細胞と，中葉の PAS 陽性細胞の萎縮も顕著であったが，他の下垂体腺細胞には，明瞭な変化が認められなかった．

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