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Mercurial Induced Changes in the Hypothalamo-Neurohypophysical Complex in Relation to Reproduction in the Teleostean Fish, *Channa punctatus* (Bloch)

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In recent years, rapid progress achieved in industrial and agricultural sectors resulted in widespread mercury contamination of water, due to indiscriminate use of mercurial fungicides in agriculture and discharge from the industrial wastes and factory effluents into rivers. Fish are known to accumulate the highest concentration of mercury in different organs including brain (UNEP/WHO 1976; Hawryshyn and Mackay 1979). In general, mercurials are well-recognized neurotoxins inducing neuronal necrosis and constitute an important gamut of pollutants because of their high toxicity; immutable, nonbiodegrable and persistent nature and tendency to undergo food chain biomagnification (Takeuchi 1972; Vallee and Ulmer 1972). In addition, the inorganic form of mercury is also reported to be nephrotoxic in mammals (Klue 1982) as well as in fish (Kirubagaran and Joy 1988).

Although extensive work has been done on neurotoxic, teratologic and genetic effects of mercurials in a variety of animals including man (Clarkson 1972; UNEP/WHO 1976; Clarkson and Marsh 1982), very little is known about their impacts on the neuroendocrine physiology of reproduction. The available investigations largely pertain to mammals (Lamperti and Printz 1973; 1974; Lamperti and Niewenhuis 1976). However, reports on the toxic effects of longterm exposure to mercury compounds on physiology of reproduction are meagre in fish (Ram and Sathyanesan 1983, 1986). In the present communication, inorganic mercuric chloride ($HgCl_2$) and organic mercurial fungicide (Emisan) induced changes in the hypothalamo-neurohypophysial system in relation to reproduction of C. punctatus are described after chronic exposure for 6 months.

MATERIALS AND METHODS

Over one hundred fifty adult C. punctatus, weighing 50±5g and measuring 14±2cm in length, used in this investigation were bought from local fish market at Varanasi. They were acclimated to the laboratory conditions for two weeks prior the experimentation. Fish were divided into three equal groups of 50 each and kept in 80-L glass aquaria containing chlorine free well-

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water of pH 7.2, hardness 154 ppm (as $CaCo_3$), alkalinity 68 ppm (as $CaCO_3$), dissolved oxygen 7.2 ppm and conductivity 0.56 mMhos. The aquaria were kept under ambient natural light and temperature conditions. The average water temperature from January to June was 20,25,27,30,33, and 36°C, respectively.

Fish in group I & II were exposed to 'safe concentrations' 0.01 ppm of inorganic mercuric cholride ($HgCl_2$) and 0.20 ppm of an organic mercurial fungicide, Emisan (Methoxy Ethyl Mercuric Chloride; MeEHgCl, bought from Exel India Ltd.), respectively. The concentration of mercurial compound in which the fish lived apparently normally during 96 hr of exposure was identified as toxicologically safe dose, as described by Basak and Konar (1977). The untreated group III served as the control. Aquaria water containing the compounds were changed on alternate days after feeding the fish with minced goat liver ad libitum. The experiment was started in the first week of January, when the gonads were in immature stage-I condition, and terminated after a continuous exposure for 6 months in the last week of June, when the gonads in control were fully matured. During the experiment, fish is $HgCl_2$-treated group did not show any apparent signs of toxicity. However, some of Emisan-exposed fish exhibited obvious blanching of the skin which is indicative of its toxicity. At the end of the experiment, fish were sacrificed by decapitation and the required tissues were dissected and processed. The gonads and the pituitaries, intact with brain, of 10 specimens from each group were fixed in Bouin's fluid and Bouin's sublimate, respectively. Paraffin sections were cut at 5 µm thickness, and the gonads were stained with hematoxylin using eosin as counter stain. Pituitaries were stained with lead hematoxylin-periodic acid-Schiff-Orange G (PbH-PAS-OG), Alcian Blue (AB)-PAS-OG and Gomori's Aldehyde Fuchsin (AF) using Halmi's counter stain. The brain monoamine oxidase (MAO) activity was also estimated adopting the enzyme isotopic technique of Parvez and Pervez (1973) and expressed in nanomoles amine deaminated/milligram tissue/hour $x10^{-2}$. Significance of the data was calculated using Student t-test.

RESULTS AND DISCUSSION

In teleosts, studies on the hypothalamic-neurohypophysial control of reproduction are still in progress (Peter 1983; DeLeeune 1985; Peter et al. 1986). So far, the variable results concerning the locolization of different nuclear centres in the brain responsible for secretion of gonadotropin releasing hormone (GnRH) have been reported, which may be due to species differences (DeLeeune 1985). In the African catfish, Clarias gariepinus (DeLeeune et al. 1985) and in C. punctatus (Haider and Sathyanesan 1972), it has been investigated that probably the majority of the nucleus preopticus (NPO) neurons are responsible for GnRH secretion which, in turn, regulates synthesis and release of gonadotropin(s) in the pituitary.

In C. punctatus, the dorsal half of the nucleus preopticus

(NPO) which is formed of larger neurons is called the pars magnocellularis (PMC) and ventral half constituted by smaller neurons is pars parvocellularis (PVC). The NPO is situated on either side of the third ventricle, and its neurons give rise to the left and right preoptic neurophypophysial neurosecretory tracts which join together prior entering the pituitary stalk . The nuclei of neurons are stainable by AB-PAS-OG procedure with light cytoplasmic area, but their neurosecretory material (NSM) stains darkly with AF. In the control fish, the NPO neurons were large, actively secreting characterized by their prominent rounded nuclei and median nucleoli (Fig.1) with adequate quantity of AF-positive NSM in the perikarya (Fig.4). In fish of both experimental groups, the neurons were smaller, inactive, containing scanty quantity of NSM and exhibited various degrees of degeneration. In those exposed to $HgCl_2$, many neurons were showing cellular pyknosis and nuclear necrosis leading to drop out (Fig.2). However, a few viable neuron with moderate quantity of NSM was also seen (Fig.3). On the other hand, in MeEHgCl-experimental fish the degenerative changes were more pronounced, as most of the neurons were pyknotic and necrotic (Figs. 3, 6). Corresponding with nuclear changes in the neurons, the brain MAO activity (mean±SD of five fish in each group) was also significantly inhibited in $HgCl_2$ (1.782±0.105, $P < 0.05$) and MeEHgCl (1.808±0.106, $P < 0.05$) treated groups as compared with control (2.051±0.132).

The teleost C. punctatus is a seasonal breeder and spawns during monsoon months in late June to July. In the present study, the gonadal maturation of fish exposed to safe concentrations of $HgCl_2$ (Ram and Sathyanesan 1983) and MeEHgCl fungicide (Ram and Sathyanesan 1986) was significantly inhibited. The ovaries from both experimental fish were in immature stage-I condition, totally devoid of vitellogenic oocytes, whereas in the control they were fully ripe with majority of vitellogenic matured stage-IV oocytes. In the testis of treated fish, sperms were lacking and the Leydig (interstitial) cells were inactive and atrophied. In the control, the seminiferous tubules were filled with sperm masses and Leydig cells were large with prominent rounded nucleus and nucleolus. Corraborating with gonadal growth cycle, changes in the gonadotrophs of pituitary were also observed. In the control fish having mature gonads, the proximal pars distalis (PPD) was dominated by large actively secreting, hypertrophied vacuolated gonadotrophs, whereas in the pituitaries from fish of both experimental groups the gonadotrophs were small, inactive involuted and fewer in number. These results suggest that mercurials may have been inhibited the gonadal growth through impairing the pituitary-gonadal axis.

In mammals, mercury is known to react with sulfhydryl (-SH) group on the cell membrane causing impairment of active transport and cell function (Passow et al. 1961). In hamster, mercury causes inhibition of follicular maturation, morphological prolongation of the corpora lutea and fluctuation in the level of pro-

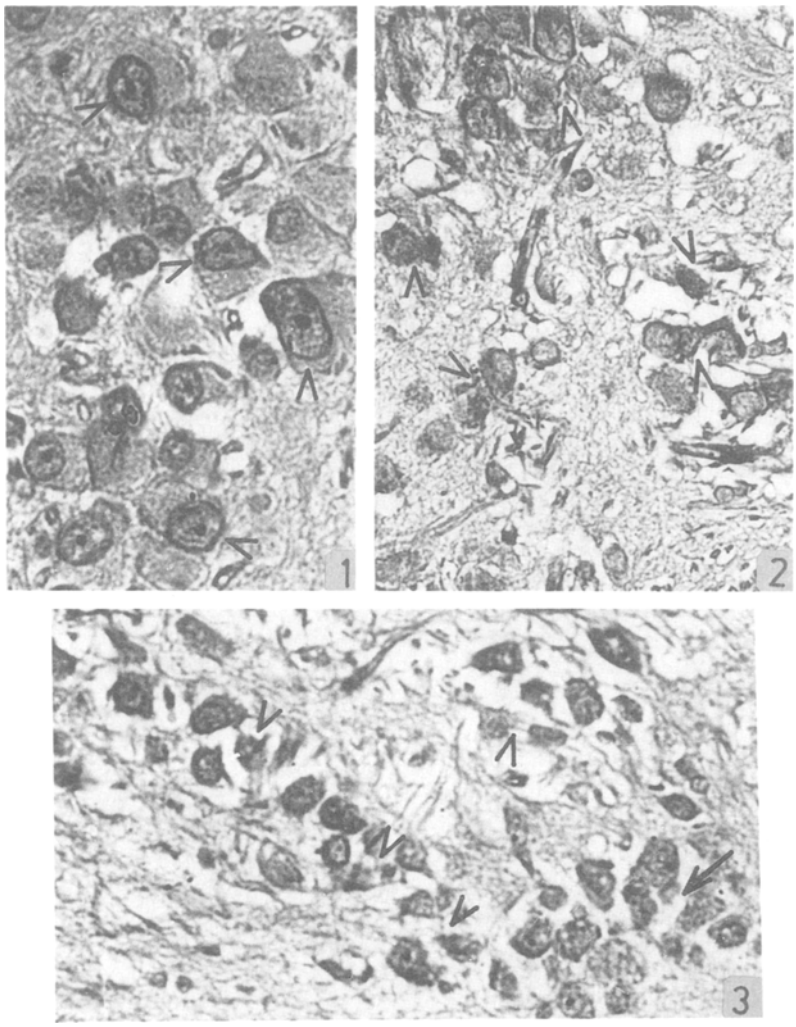

Figure 1. The pars magnocellularis (PMC) part of the nucleus preopticus (NPO) of control fish, showing large active neurons with prominent nuclei and nucleoli (arrowheads). AB-PAS-OG, X 600.

Figure 2. Same region as in Fig. 1 of $HgCl_2$-treated fish, showing small inactive neurons. Arrowheads indicate degenerating neurons with necrotic nuclei. AB-PAS-OG, X 600.

Figure 3. The PMC part of MeEHgCl-exposed fish, showing groups of neurons in different stages of degeneration (arrowheads). Arrow indicates debris of completely dissoluted neurons. AB-PAS-OG, X 600.

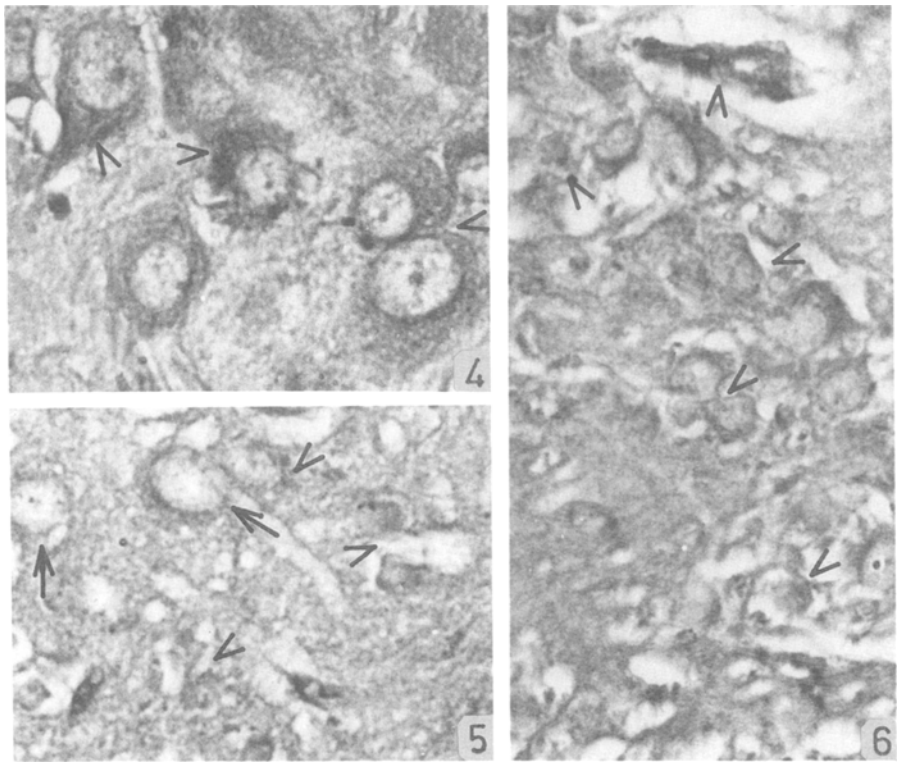

Figure 4. The PMC neurons of control fish, showing AF- positive neurosecretory material (NSM) in the perikarya (arrowheads). AF with Halmi's counter stain, X 750.

Figure 5. The neurons of same region as in Fig. 4 of $HgCl_2$-treated fish. Arrowheads indicate degenerating neurons devoid of NSM. A few viable neurons can also be seen (arrows). AF with Halmi's counter stain, X 750.

Figure 6. The PMC part of MeEHgCl-exposed fish, showing debris of degenerated neurons separated by empty spaces (arrowheads). AF with Halmi's counter stain, X 750.

gesterone in the plasma and corpora lutea (Lamperti and Printz 1973). Further, Lamperti and Printz (1974) found the accumulation of mercury in the corpora lutea, the sinusoids of the pituitary and selectively in the cytoplasm and axoplasm of some neurons in the hypothalamus including the arcuate nucleus which is responsible for synthesis and release of GnRH. Later, Lamperti and Niewenhuis (1976) reported the involvement of mercury in retarding ovarian growth through the hypothalamo-hypophysial-ovarian axis directly by impairing the responsiveness

of ovary and pituitary to hormonal stimulation, and inhibiting the synthesis and release of GnRH from the arcuate neurons. In fish Clarias batrachus exposed to lead nitrate for 5 months, Katti and Sathyanesan (1986) reported degeneration of the NPO neurons and inhibition of gonadal maturation possibly mediated through the hypothalamo-hypophysial-gonadal axis. In the present study, the adverse changes observed in the gonadotrophs and the NPO neurons of fish exposed to both $HgCl_2$ and MeEHgCl suggest that mercurials might have inhibited the secretion of GnRH from the NPO which, in turn, caused inactivation of the pituitary gonadotrophs resulting in retardation of gonadal maturation. However, more pronounced deleterious changes in the NPO neurons of MeEHgCl-experimentals may be due to increased accumulation of this organic mercury form than inorganic one. Berlin et al.(1969) and Magos (1968) reported that biological cell membrane and the blood-brain-barrier discriminate against ionic and inorganic mercury, but permit more passage of organic mercury due to its high lipid solubility (Clarkson 1972; Vallee and Ulmer 1972).

MAO is a FSH dependent (Urry et al.1974) mitochondrial enzyme responsible for oxidative deamination of monoamines. In the rat, based on the inverse correlation between gonadotropins release and dopamine levels, Srivastava et al. (1980) suggested that MAO may be playing an important role in the dopaminergic control of gonadotropin release. In an earlier study on C. punctatus, Ram and Sathyanesan (1985) have reported that exposure for 80 days to sublethal dose of mercurials, $HgCl_2$ and MeEHgCl, caused inhibition of brain MAO activity with accompanying retardation of gonadal growth. In the present investigation, both mercurials induced reduction in MAO is also an indirect evidence of impairment of the monoaminergic system responsible for modulating the hypophysiotrophic control of gonadotropic function.

Thus, on the basis of these results, it can be inferred that mercurials induced inhibition of the gonadal growth might have been mediated through the impairment of the hypothalamo-neurohypophysial-gonadal axis, in this species.

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