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Serotonin and MSH Secretion: Effect of Parachlorophenylalanine on the Pituitary Cytology of the Eel

Madeleine Olivereau

Laboratoire de Physiologie de l'Institut Océanographique, Paris, France

Summary. Parachlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase which depletes brain serotonin in higher vertebrates, was injected into freshwater eels. After 4 or 6 injections (200 mg/kg/day) or 10 injections (100 and 140 mg/kg/day), the animals are paler, with a low melanophore index. In the pituitary gland, granules tend to accumulate in the basal part of the MSH cells and in the perinuclear area. Cells appear smaller with a decreased nuclear area ($P<0.001$). In the neurohypophysis, the amount of neurosecretory material is often reduced. Conversely, injections of 5-hydroxytryptophan induce a strong darkening, a result similar to that previously reported in some amphibian species and in one lacertilian species. These data substantiate the hypothesis of a stimulatory influence of 5-hydroxytryptamine on MSH release and possibly its synthesis in the eel and other lower vertebrates.

Key words: Parachlorophenylalanine – Serotonin – MSH – Pituitary gland – Eel.

Introduction

The secretion of a pituitary melanophore-stimulating hormone (MSH) is under the control of the hypothalamus. Some hypothalamic peptides or some sequences of neurohypophysial hormones may act as a MSH release stimulating factor (MRF) and as a MSH release inhibiting factor (MIF) in a few mammals (Hadley and Hruby, 1977). Neither α-MSH nor MIF affects the amount of serotonin or 5-methoxytryptamine in the brain of intact rats, but do reduce serotonin

Send offprint requests to: Prof. Madeleine Olivereau, Laboratoire de Physiologie, Institut Océanographique, 195, rue Saint-Jacques, 75005 Paris, France

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accumulation in the frontal cortex of hypophysectomized rats (Spirtes et al., 1975). The effect of MIF and MRF, still controversial in reptiles (Thornton and Geschwind, 1975) and amphibians (Hadley and Hruby, 1977), has not yet been investigated in fish.

Furthermore, dopamine, a neurotransmitter, also appears to be involved in this particular hypothalamic control in vertebrates, including fish. In the eel, dopamine lowers MSH release (Olivereau, 1975). Conversely, this release is stimulated by drugs reducing brain catecholamines, such as reserpine (Olivereau, 1972; Fremberg and Meurling, 1975) or 6-hydroxydopamine (Fremberg and Olivereau, 1973). Likewise, it is increased by pimozide, as shown by the pituitary cytology and the increase of the melanophore index (MI) value in the eel (Olivereau, 1978b). Pimozide is considered to block selectively dopaminergic receptors (Janssen et al., 1968); however, recent results (Grabowska, 1976, Smythe, 1977) suggest that pimozide simultaneously reduces the utilization of brain serotonin in the rat.

Thus, neurotransmitters other than dopamine appear capable of interferring with MSH secretion, at least in the rat (Kastin et al., 1969). Among them, 5-hydroxytryptamine (5-HT), implanted in the third ventricle, depletes pituitary MSH in the rat (Taleisnik and Celis, 1972). Injected into the rat, various substances, including serotonin, do not increase the pituitary MSH content; in fact, some depletion of MSH was noted in most cases (Schally and Kastin, 1966). In vitro, only dopamine is capable of counteracting the stimulatory effect of dibutyryl cyclic AMP on the MSH release in the rat pituitary. MIF, MRF and serotonin, among other factors, have no effect (Baker, 1976).

In reptiles, subcutaneous injection of serotonin creatinine sulfate or its precursor, 5-hydroxytryptophan (5-HTP), darkens green lizards, *Anolis carolinensis*. In vitro, 5-HT or 5-methoxytryptamine greatly stimulates MSH release from single incubated hemi-pituitaries. Neither 5-HT nor 5-HTP causes darkening of isolated *Anolis* skin (Thornton, 1974a and b; Thornton and Geschwind, 1975). MIF and MRF are without effect both in vivo and in vitro.

In the frog (Davey, 1960), indolealkylamines, tryptamine and serotonin exert a skin darkening effect. Likewise, 5-methoxytryptamine promotes MSH release in the mouse or frog pituitary in vitro (Bower, 1974). The hypothesis that 5-HT, detected in *Xenopus* skin (Van de Veerdonk et al., 1961), mediates the activity of MSH on the melanophores was proposed by Davey (1960). Most probably, MSH acts by increasing the level of cAMP in the melanophore.

When tested in a selachian, the dogfish, *Scyliorhinus canicula*, 5-HT injected into the cranial fluid, had not effect on the melanophores of either pale or dark fish (Wilson and Dodd, 1973).

Almost no data are available on a possible serotoninergic mechanism involved in MSH release in teleost fish. Parachlorophenylalanine (pCPA), a drug which blocks the activity of tryptophan hydroxylase, is a useful tool to investigate this point. It inhibits the synthesis of 5-HT in mammals (Koe and Weissman, 1966), resulting in a depletion of pineal and cerebral serotonin. Injected into the eel, it reduces the granule release in prolactin-secreting cells (Olivereau, 1978c) and induces a decrease of the MI (Olivereau, 1975). It thus appeared necessary to search for a possible effect of pCPA on the MSH-secreting cells.

Materials and Methods

Male silver eels, *Anguilla anguilla* L., were kept under controlled conditions: 20 ± 1° C, photoperiod 12 h light: 12 h dark (light 8–20 h) in aerated, dechlorinated tap water on a green background.

A first group of 14 eels (average weight 65 g) was injected daily into the body cavity with the dl-pCPA methyl ester hydrochloride (Sigma). They were treated for 3 or 5 days (4 or 6 injections) with 12.5 mg/day (200 mg/kg) and sacrificed 2 to 4 h after the last injection, being anesthetized with MS 222. Two eels received only 2 injections. Seven eels (90–110 g) were treated for 8 days with 17 mg/day (170 mg/kg). The MI was rapidly evaluated before anesthesia, as MS 222 may induce a slight melanodispersion increasing with time. At autopsy, some small hemorrhages were noted in the body cavity, at the site of injection; they may possibly be correlated to the low pH (2.5) of the solution of pCPA and to some effect on blood platelets, as has been described in mammals.

Two years later, another group of 8 eels (average weight 50 g) was injected with dl-pCPA (Sigma) finely ground and suspended in NaCl 0.6 % solution and a trace amount of Tween 80. This suspension was injected daily at a dose of 5 mg for the first 5 days, and 7 mg for the last 5 (100 and 140 mg/kg). Fourteen control eels received either an acid solvent or the NaCl + Tween 80 vehicle in the same year as the treated eels.

The pituitary gland was rapidly immersed in sublimated Bouin-Hollande fixative, embedded in paraffin and sectioned at 4 µm. Sections were mainly stained according to the Herlant tetrachrome method and with lead-hematoxylin associated or not with the PAS technique. Nuclear areas were measured using a planimetric technique and statistical calculations (Student's t test) were made as previously reported (Olivereau, 1978a). The neurosecretory material (NSM) was demonstrated by means of the aldehyde-fuchsin technique of Gomori, after permanganate oxidation, according to Gabe (1953).

Results

1. Melanophore Index (MI)

In control fish, MI values are rather high and similar in both groups (4.4 and 4.3).

Eels treated with pCPA for one or two days do not appear paler, but on the third day, some lightening is detected and on the fourth day, the MI value is significantly reduced (1.9). This decrease remains evident after five (1.6) or nine days (1.3). Only one fish out of seven did not turn pale after six injections and remained similar to the control fish. Eels treated for eight days (170 mg/kg) had low MI values (1 to 1.1).

2. MSH-Secreting Cells

MSH cells (previously described by Olivereau, 1978a) are strongly stained with lead-hematoxylin. Their functional significance has been confirmed by immunocytochemical techniques (Olivereau et al., 1976). Their nuclear area is larger in the second group than in the first one, in spite of an almost similar MI. In both groups, they are moderately granulated (Fig. 2). Nucleoli are often visible in a clear karyoplasm.

After two injections, the amount of granulation increases, mainly in the distal part of the cell (Fig. 3). After four injections, the decrease of the nuclear area in barely significant ($P = 0.05$) and some cells are still well granulated (Fig. 4). After six

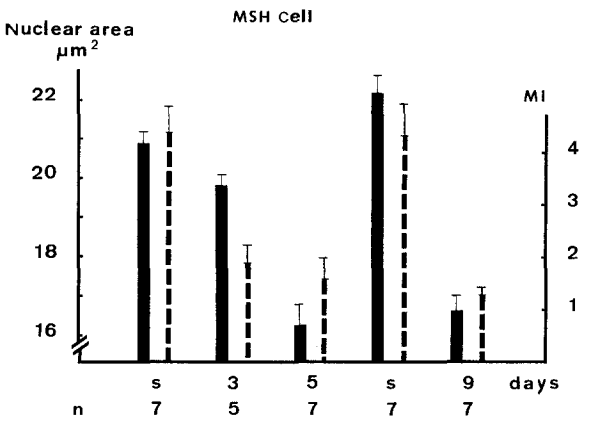


Fig. 1. Variations of nuclear area of the MSH cells (left, solid bars) and melanophore index (right, dashed bars) in eels treated with pCPA. *S* control eels injected with the solvent compared with eels receiving 4, 6 and 10 injections within 3, 5 and 9 days, respectively; *n* number of fish. Mean and standard error of the mean

or ten injections, the nuclear area is markedly reduced ($P < 0.001$) (Figs. 1, 4 and 5). The nucleolus is smaller and less prominent in a darker karyoplasm (Fig. 5). The cell volume decreases. This atrophy is quite evident in the perinuclear cytoplasm, where an ergastoplasmic area is not detectable (Fig. 5). The elongated processes reaching the neurohypophysial tissue or the connective limiting membrane are often shorter or thinner. After six injections, granule storage in the distal part of the cell is less evident, but it extends to the perinuclear area. The granule accumulation does not further increase in eels treated for nine days (140 mg/kg/day) (Fig. 6), and granule synthesis also seems reduced.

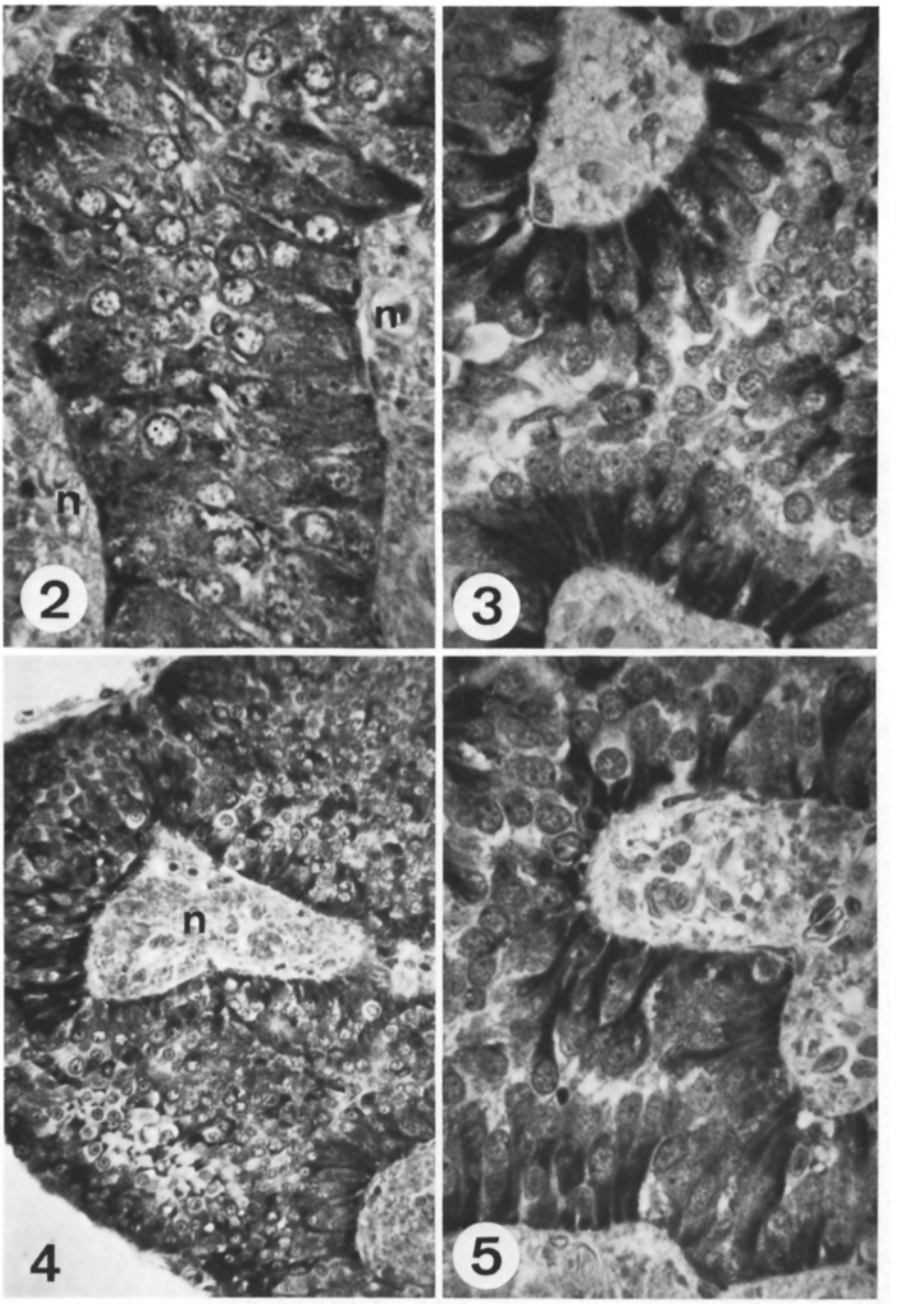
3. PAS-Positive Cells

This second cell category of the pars intermedia, the functional significance of which remains unknown, is quite variable in number among control eels, mainly in the second experiment.

In pCPA-treated fish, these cells tend to become larger, with some processes extending toward the neurohypophysial border. These processes are well granulated and are intermingled among those of the lead-hematoxylin-positive cells (Fig. 6). However, this reaction remains discreet compared with the response of eels kept in deionized water (Olivereau, 1967), and neither mitotic activity nor degranulation can be detected. No significant variation occurs in the sodium, potassium, calcium and chloride plasma levels of the treated animals.

4. Hypothalamo-Neurohypophysial Neurosecretory Material (NSM)

In the neurohypophysis, the amount of aldehyde-fuchsin-positive NSM appears reduced in most of the eels treated with pCPA for three or five days (Figs. 7 to 9). Its



Figs. 2–5. Pars intermedia of the eel stained with lead-hematoxylin

Fig. 2. Control eel. A ramification of the pars intermedia surrounded by neurohypophysial tissue (*n*). ×930

Fig. 3. pCPA-treated eel, 2 injections. Accumulation of granules in the basal part of the MSH cells. Some PAS-positive cells, which appear unstained, are located in the center of the strand. ×930

Fig. 4. pCPA-treated eel, 4 injections. MSH cells are well granulated and are in striking contrast to unstained PAS-positive cells either in the middle or the periphery of ramifications of the pars intermedia. ×370

Fig. 5. pCPA-treated eel, 10 injections. The nuclear and perinuclear areas are reduced. Granules still accumulate in slender and often shorter cell processes. A few PAS-positive cells are closer to the neurohypophysial tissue. ×930

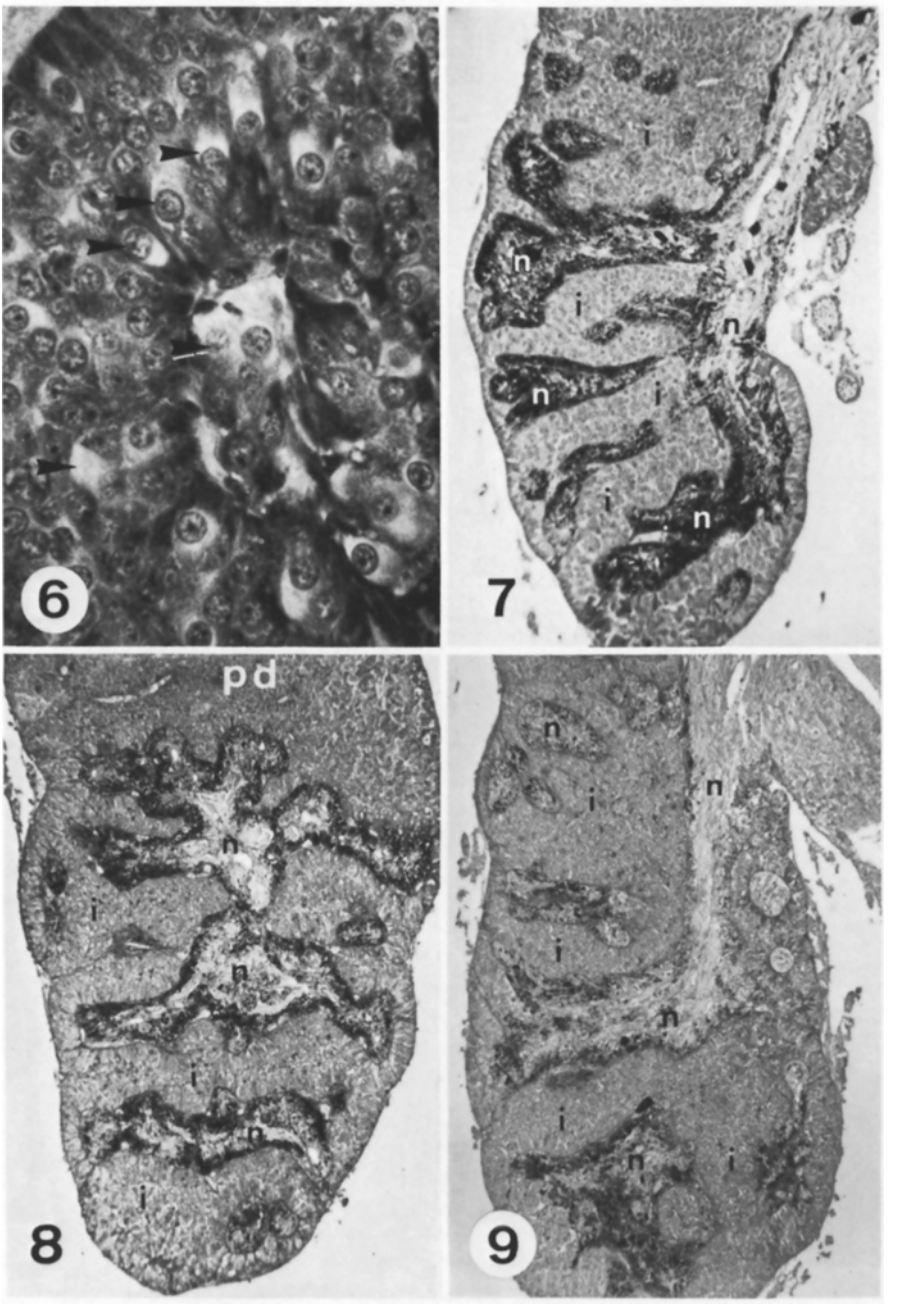


Fig. 6. pCPA-treated eel, 6 injections. PAS-positive cells (*arrowhead*) are more peripherally situated and appear more or less stimulated. ×930

Figs. 7–9. Ramifications of the neurohypophysis (*n*) extending into the pars intermedia (*i*). Aldehyde-fuchsin. ×140 **Fig. 7.** Control eel. **Figs. 8 and 9.** Eels treated with 4 and 6 injections of pCPA, respectively, showing a progressive reduction of the neurosecretory material; *pd* proximal pars distalis

distribution is modified: the NSM is often dispersed in finer granulations; it is less frequently observed in close contact with the connective-tissue layers delimiting the pars intermedia and the adjacent blood vessels. After ten injections (lower dose), the reduction of NSM is not apparent in four out of seven eels.

In the magnocellular area of the preoptic nucleus, the NSM is slightly reduced after four injections. However, this is no longer evident after six or ten injections. The amount of NSM is not modified in the main preoptico-neurohypophysial tract near the recessus infundibularis and in the axons located in the various tracts emerging from the preoptic nucleus.

Discussion

The decrease of MI values in pCPA-treated eels seems to be correlated to a blockade of MSH release by the pituitary. A similar inhibition has already been demonstrated in the rat: a hypertonic saline solution induces a depletion of pituitary MSH which can be prevented by a pretreatment with pCPA or methysergide, an antiserotoninergic drug, injected into the third ventricle (Taleisnik and Celis, 1972).

The reduction of the neurohypophysial NSM is not clearly correlated with a modification of the preoptic nucleus. As no axonic accumulation of NSM can be detected, NSM transport does not seem to be affected. Initially, a reduced synthesis in the nerve cell bodies is probable, but does not appear evident after six or then injections. These results differ partly from those obtained in the rat: within 24 h, a single injection of pCPA produces an incomplete depletion of the NSM in the supraoptic nuclei; it induces a large accumulation of NSM in the axonic processes and in the median eminence, which increases after 48 h. This result suggests a disturbance in axonic transport of the NSM (Tangapregassom et al., 1975), but this hypothesis does not seem to apply to the eel treated for a longer period of time with similar or smaller doses.

That pCPA administered into the body cavity of eels may act on brain indoleamines is most probable, as in mammals it is often injected via the intraperitoneal route or absorbed per os in human subjects. For example, in the rat, two doses of 316 mg/kg of pCPA methyl ester HCl induce a 83 % depletion of brain serotonin (Van Delft et al., 1973). It may act on the brain and/or the pituitary which contains, at least in the rat, a high amount of serotonin, mainly in the intermediate lobe. This pituitary 5-HT level is increased by melatonin (Piezzi and Wurtman, 1970). A participation of the pineal gland which elaborates 5-HT cannot be excluded, but a hypersecretion of MIF or a reduced secretion of MRF remains hypothetical in the eel.

The eel brain certainly contains serotoninergic neurones according to microspectrofluorimetric studies (Fremberg et al., 1977b), although dopamine predominates. Some serotoninergic nerves are situated in the walls bordering the third ventricle (Fremberg et al., 1977a). It may be supposed that hypothalamic and pituitary serotonin depletion prevents or reduces MSH release, agreeing with the low MI values of the treated eels. After nine days, granule storage does not increase and MSH synthesis may also be reduced, as the cells and the nuclei slowly atrophy in most of the treated fish.

Confirmation of these results was obtained by injecting 5-HTP: eels rapidly darken and MSH cells were strikingly stimulated, as evidenced by ultrastructural observation of increased synthesis and release (Olivereau et al., 1977, unpublished data). However, other mechanisms, mainly dopaminergic, and perhaps systems still unidentified in teleosts, may interfere in controlling MSH release and/or synthesis. Furthermore, some antiserotoninergic drugs do not always induce a paling effect (unpublished result).

That the pituitary gland is implicated in the response to pCPA seems probable as 5-HTP is no longer effective in hypophysectomized eels (unpublished data). In the case of a pCPA treatment, there is no point in injecting hypophysectomized eels; the MI value is already very low (around 1).

These results obtained with pCPA, corroborated by those of 5-HTP-treated eels, substantiate the hypothesis of a serotoninergic mechanism controlling MSH release, although its effect may be less intense than that of the dopaminergic control. In addition, pCPA may have some effect on dopaminergic regulation (Koe and Weissman, 1967). The stimulatory serotoninergic control of MSH release appears similar to that previously described in some amphibian and at least one reptilian species.

The apparent antagonism between dopaminergic and serotoninergic mechanisms modulating MSH release appears to agree with data obtained on the autotransplanted pituitary of several teleost species. When the pituitary gland is disconnected from the brain, the suppression of the dopaminergic inhibitory control does not induce a hypersecretion of MSH. Indeed, this secretion often appears reduced in Cyprinodonts (Olivereau and Ball, 1966; Kah, 1978). Among other factors still unidentified, the possible lack of serotoninergic innervation of the graft may be responsible for the low or subnormal activity of MSH cells, as in the eel (Olivereau, 1969). However, hypothalamic lesions reducing the fluorescence of the neurointermediate lobe are able to induce a darkening of the eel (Fremberg et al., 1977a and b).

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