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Different Neuronal Populations of the Nucleus Preopticus and their Response to Ovariectomy, and Oestradiol Benzoate Treatment with a Note on the Gonadotrops in the Catfish, *Clarias batrachus* (Linn.)

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A cytoarchitectonic study of the hypothalamus of the catfish, *Clarias batrachus* reveals the occurrence of two divisions of the nucleus preopticus (NPO). A neuronal group in the form of a vertical limb adjacent to the preoptic recess and another situated horizontally above the optic chiasma permit their identity as NPO-paraventricularis (NPO-P) and NPO-supraopticus (NPO-S) respectively. In addition to the above, a few neurones termed as the bridge cells occur between the two divisions. Cytoarchitectonically, the NPO-P is divisible into four subdivisions, and the NPO-S into three subdivisions.

In response to 40-day ovariectomy only the medical, lateral and posterodorsal subdivisions of the NPO-P undergo significant stimulatory changes ($P < 0.001$), while others show no remarkable changes. The gonadotrops undergo hypertrophy and hyperplasia. Following administration of oestradiol benzoate (EB) only the above three subdivisions of NPO-P show significant ($P < 0.001$) regressive changes. The gonadotrops are also regressed. It is concluded that only certain subdivisions of the NPO respond to ovariectomy and EB administration, concomitantly with the gonadotrops.

Introduction

It has been suggested that the neurohypophysial hormones of fish play a role in a variety of functions *viz.*, antidiuresis, vasopressor effects, osmoregulation (*cf.* Peter 1977) and in control of gonadal activity via gonadotropins (Stahl & Leray 1962, Dixit 1970, Prasada Rao & Betol 1973, Viswanathan & Sundararaj 1974a). It is well known that the nucleus preopticus (NPO) of fish possesses aldehyde fuchsin (AF)-positive neurones divisible into dorsally situated pars magnocellularis (PMC) composed of large neurones, and ventrally situated pars parvocellularis (PPC) consisting of small neurones (Dodd & Kerr 1963, Sathyanesan 1970, Prasada Rao & Betole 1973, Viswanathan & Sundararaj 1974a, Peter et al. 1975). It then appears perplexing as to how the two hitherto recognized subdivisions of the NPO in fish hypothalamus control varied functions cited above. An attempt has therefore, been made in the present study, to categorize the NPO neu-

rones into subdivisions based on criteria like neuronal size, shape, location, orientation, nuclear size, shape and diameter. Having identified as many as 7 subdivisions, studies are being conducted to analyze the possible functional significance of each of these subdivisions. Since the NPO has been suggested to have some role in gonadal function, different neuronal subdivisions are examined in the present study after ovariectomy and after administration of oestradiol benzoate (OB) in order to understand which of the subdivisions have some relation with the gonads in the catfish, *Clarias batrachus* (Linn.). Concomitant changes undergone by the gonadotrops have also been incorporated.

Materials and Methods

More than 80 adult female catfish, *Clarias batrachus* (60–80 g in weight, 20–24 cm in length) used in the present study were obtained from the local Telenkheri tank in the month of September. Twenty female fish were sacrificed by decapitation on the day of their collection. The brains were dissected carefully and fixed and acqueous Bouin's fluid for 24 hr. They were cut serially in transverse, sagittal and horizontal planes at 6–10 μ thickness, and stained with Kluver and Barrera technique and other techniques described in an earlier paper (Prasada Rao et al. 1972). Histological criteria such as cell area, pattern of distribution and orientation of neurones, staining intensity, nuclear diameter, nature of nucleoli etc., have been used to demarcate different subdivisions in the NPO of *Clarias batrachus*.

Catfish acclimatized to laboratory conditions for about 10 days were used in the experiments. The fish were maintained in identical glass aquaria ($60 \times 30 \times 30$ cm) containing 30 litres of tapwater and maintained under normal daily photoperiods (13L+11D) and temperature ($27 \pm 1^{\circ}$C). They were fed minced goat liver daily *ad libitum*. On the day of performing gonadectomy, eight fish were sacrificed and their brains collected and fixed. These served as initial controls. Ten fish were ovariectomized and another 10 were sham-operated as described in a previous study (Prasada Rao et al. 1972). Forty days after gonadectomy, the ovariectomized and sham-operated controls were sacrificed. Their brains alongwith pituitaries were fixed, sectioned and stained as described before.

On the day of starting EB injections, eight fish were sacrificed, and their brains with pituitaries collected for further study and these served as initial controls. Out of the remaining 20 animals, 10 were injected once a week intramuscularly with EB (Martin & Harris) dissolved in sterile olive oil at the dosage of 400 μg/100 gm body weight. Ten controls were injected with an equivalent volume of sterile olive oil. All the fish were sacrificed 40 days after commencement of injections and their brains along with pituitaries were collected and fixed for further study.

To assess the histocytological changes undergone by the NPO subdivisions and the gonadotrops after ovariectomy and EB administration the procedure given in earlier papers was adopted (Prasada Rao & Betole 1973, Prasada Rao & Subhedar 1977). As the neurones exhibit irregular outlines, the neuronal area was calculated planimetrically from camera lucida drawings. The data were analyzed statistically by Student's t test using 5% level of significance.

Results

(*A*) *Neuronal populations of NPO:* As described earlier (Sathyanesan 1970, Prasada Rao & Betole 1973) the preoptic nucleus of *Clarias batrachus* is situated on either side of the preoptic recess, slightly above and anterior to the optic chiasma. It shows a

long vertical proximal limb situated adjacent to the preoptic recess and a short distal horizontal limb located dorsal to the optic chiasma. Scattered neurones are present in-between the two limbs. In contrast to earlier studies, large neurones have been observed in the distal limb of the NPO and even in the ventral region of the vertical limb. In view of the occurrence of large neurones in the ventral region, it becomes apparently difficult to distinguish a dorsal zone of large neurones (PMC) and a ventral division of small neurones (PPC) as has been reported conventionally. The NPO of *Clarias batrachus* appears to be comparable with the nucleus preopticus pars magnocellularis described in the different fishes by Charlton (1932) in his pioneer work.

In order to bring conformity of the AF-positive neuronal groups in fish with those of mammals, birds and reptiles and to maintain uniformity in terminology, the vertical limb of NPO of *Clarias batrachus* is termed in the present study as the NPO-paraventricularis, and the horizontal limb which is immediately above the optic chiasma as the NPO-supraopticus (figure 1). In view of the disposition of the neurones of the NPO-paraventricularis in the form of vertical laminae parallel to preoptic recess, and on account of their increase in size (see table 1) from the recess lateralwards, the following four subdivisions can be recognized (figure 1):

Table 1 *Neuronal areas and nuclear diameters in different subdivisions of NPO of* Clarias batrachus

Name of the subdivision	Cell area (in μf)	Nuclear diameter (in μ)
1. NPO–P		
(i) **ppv**	30.99 ± 4.32*	4.32 ± 0.08
(ii) **pm**	77.62 ± 6.57	5.49 ± 0.12
(iii) **pl**	162.19 ± 12.23	7.54 ± 0.14
(iv) **ppd**	227.13 ± 22.12	8.57 ± 0.16
2. NPO–S		
(i) **pv**	42.47 ± 4.13	4.98 ± 0.07
(ii) **pm**	92.58 ± 7.17	6.28 ± 0.08
(iii) **pl**	211.79 ± 18.24	8.34 ± 0.12

NPO-P : NPO-paraventricularis; NPO-S : NPO-supraopticus; pv, pars ventralis; ppv, pars periventricularis; pm, pars medialis; pl, pars lateralis; ppd, pars posterodorsalis. *Means ± standard error; NS, not significant.

(*i*) NPO-paraventricularis pars periventricularis (NPO-P ppv): The neurones of this subdivision are adjacent to the ventricle and are smallest in size and disposed vertically.

(*ii*) NPO-paraventicularis pars medialis (NPO-P pm): The neurones of this subdivision are moderate in size and situated lateral to the previous subdivision.

(*iii*) NPO-paraventricularis pars lateralis (NPO-P pl): The neurones in this subdivision are fairly large and form a vertical lamina of single layered cells.

(*iv*) NPO-paraventricularis pars posterodorsalis (NPO-P ppd): The neurones in this subdivision are largest in size and occupy a posterodorsal region of the NPO.

The neurones of the NPO-supraopticus are distinguishable into the following three

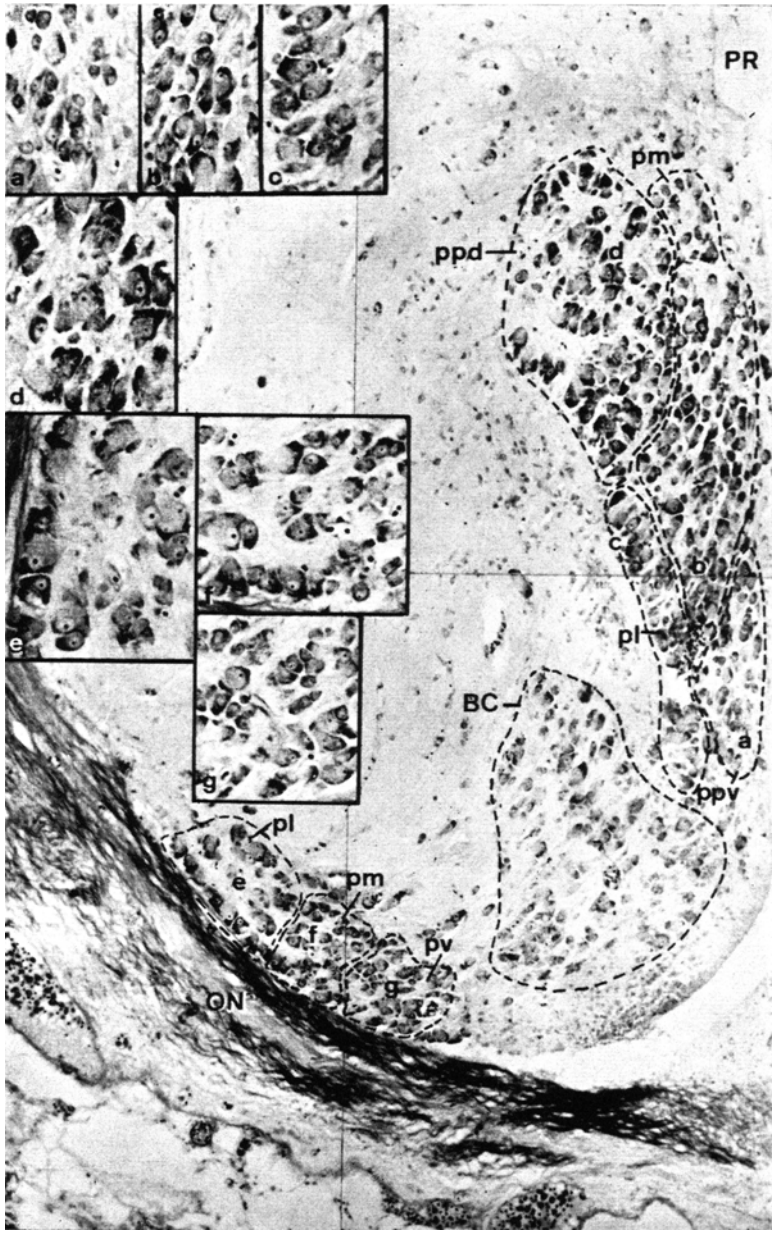

Figure 1 Photomontage of T.S. of the brain through preoptic region showing subdivisions of the NPO. The vertical limb adjacent to preoptic recess (PR) represents the NPO-paraventricularis and the horizontal limb dorsal to the optic nerve (ON) represents the NPO-supraopticus. In between these two divisions are the bridge cells (BC) (× 90).
Insets a–g represent the magnified view of the corresponding regions of various subdivisions of the NPO (× 240) (*Kluever and Barrera technique*)

subdivisions disposed horizontally.

(*i*) NPO-supraopticus pars ventralis (NPO-S pv): This subdivision consists of small neurones situated slightly away from the ventricle and occupies the ventralmost region of the brain.

(*ii*) NPO-supraopticus pars medialis (NPO-S pm): The neurones of this subdivision are of moderate size and occupy the central region.

(*iii*) NPO-supraopticus pars lateralis (NPO-S pl): The neurones of this subdivision are large and possess large cell nuclei.

Extending between the NPO-paraventricularis and NPO-supraopticus there are a number of neurones of varying sizes and termed as bridge cells.

(*B*) *Effect of ovariectomy:* The changes in the nuclear diameter in various subdivisions of the NPO are summarized in table 2. Only the medial, lateral and the posterodorsal subdivisions of the NPO-paraventricularis undergo significant hypertrophic changes after ovariectomy.

Table 2 *Effect of ovariectomy and administration of oestradiol benzoate on nuclear diameters (in micra) of various subdivisions of the nucleus preopticus*

Name of the subdivision	Effect of ovariectomy				Effect of oestradiol benzoate (EB)			
	Initial control	Sham-operated	Ovariectomized	P-value	Initial control	Oil-injected	EB-injected	P-value
1. NPO-P								
(i) ppv	4.42 ± 0.14*	4.37 ± 0.15	4.51 ± 0.17	NS	4.50 ± 0.14	4.45 ± 0.16	4.25 ± 0.16	NS
(ii) pm	5.53 ± 0.16	5.57 ± 0.12	5.84 ± 0.11	$P < 0.001$	5.63 ± 0.18	5.60 ± 0.17	5.03 ± 0.13	$P < 0.001$
(iii) pl	7.54 ± 0.20	7.52 ± 0.19	8.09 ± 0.22	$P < 0.001$	7.70 ± 0.19	7.82 ± 0.19	6.66 ± 0.17	$P < 0.001$
(iv) ppd	8.68 ± 0.20	8.59 ± 0.19	9.12 ± 0.18	$P < 0.001$	8.72 ± 0.22	8.89 ± 0.32	7.61 ± 0.18	$P < 0.001$
2. NPO-S								
(i) pv	4.39 ± 0.11	4.45 ± 0.13	4.53 ± 0.14	NS	4.55 ± 0.12	4.67 ± 0.13	4.45 ± 0.12	NS
(ii) pm	6.24 ± 0.19	6.18 ± 0.17	6.33 ± 0.17	NS	6.28 ± 0.24	6.36 ± 0.22	6.12 ± 0.18	NS
(iii) pl	8.03 ± 0.23	8.32 ± 0.21	8.01 ± 0.19	NS	8.10 ± 0.25	8.32 ± 0.20	8.18 ± 0.15	NS

NPO-P : NPO-paraventricularis; NPO-S : NPO-supraopticus; pv, pars ventralis; ppv, pars periventricularis; pm, pars medialis; pl, pars lateralis; ppd, pars posterodorsalis. *Means ± standard error; NS, not significant.

In the initial control and 40-day sham-ovariectomized fish, the neurones of the pl, pm and ppd subdivisions of NPO-paraventricularis are polymorphic and the perikarya are stained brightly except those of the ppd, which are moderately stained. The nuclei of the neurones in the pm and pl are round, whereas those of the ppd are oval or polymorphic. All the neurones possess prominent nucleoli which are centrally situated. Forty days after ovariectomy, the neurones of the above subdivisions appear large and the perikarya are weakly stained. The nuclei are deeply stained, attain a turgid and spherical nature and are eccentric in position. The nuclei of the three subdivisions become

significantly larger ($P < 0.001$) than those in the respective subdivision in the sham-operated control fish (table 2).

In the initial control fish and in the 40-day sham-ovariectomized ones the gonadotrops are moderately stained with periodic acid Schiff's (PAS) reagent and alcian blue. Forty days after ovariectomy the cyanophils are hypertrophied and show even hyperplastic changes. Some of the cells are degranulated. The vesicular nuclei ($4.32 \pm 0.12\ \mu$ in diameter) are significantly hypertrophied ($P < 0.001$) as compared to those of the control fish ($3.92 \pm 0.10\ \mu$ in diameter).

(*C*) *Effect of oestradiol benzoate injections:* The responses exhibited by the neuronal nuclei of various subdivisions of the NPO to administration of EB are summarized in table 2. Since the changes observed are limited to the pl, pm and ppd of the NPO-paraventricularis, the cytomorphological features of only these 3 subdivisions are described below.

In the olive oil-injected control and as well as in the initial control fish, the perikarya of the pm, pl and ppd of the NPO-paraventricularis are brightly stained with AF, and the nuclei show prominent nucleoli. As a result of EB administration for 40 days, the neurones of all the three subdivisions show a shrunken appearance and the perikarya are stained weakly with AF. The nuclei are reduced in size and are significantly smaller ($P < 0.001$) than those of the respective subdivisions in the vehicle-injected controls (table 2). The nucleoli could not be visualized in many neurones, whereas in others they are not prominent.

The gonadotrops of the initial control fish and those of the olive oil-injected fish are cyanophilic in nature and are moderately stained with AF, PAS and alcian blue techniques. Forty days after EB injections the nuclei in the cyanophils show a weakly stainable material in the area adjacent to the nuclei. The gonadotrops present a shrunken appearance, the nuclei are reduced in size ($3.72 \pm 0.09\ \mu$ in diameter) and are significantly smaller ($P < 0.001$) than those in the control fish ($3.96 \pm 0.07\ \mu$ in diameter).

Discussion

The cytoarchitectonic study of preoptic area of the hypothalamus reveals the existence of a supraoptic and a paraventricular group of neurones distinguishable on the basis of their location just above the optic chiasma and adjacent to the preoptic recess, respectively. In their localization these subdivisions are apparently comparable to the supraoptic and paraventricular nuclei of the higher vertebrates. Palay (1960) has pointed out that the Gomori-positive NPO pars magnocellularis of fish is homologous with the supraoptic nucleus and paraventricular nucleus of higher vertebrates. In view of the existence of a separate subdivision of AF-positive neurones dorsal to the optic chiasma, it appears justified to treat it as a subdivision of the NPO and name it as NPO-supraopticus. However, one must be cautious in assigning such an independent status to the subdivision merely because of similarity in position, and its final identification should depend upon further investigation on its functional significance.

While corroborating the earlier view that the nucleus preopticus undergoes changes following gonadectomy, administration of steroids or inhibitors of gonadal steroids (Dixit 1970, Prasada Rao & Betole 1973, Viswanathan & Sundararaj 1974b), the present study shows for the first time that only some divisions viz., NPO-P pm, NPO-P pl and NPO-P ppd undergo changes, whereas the remaining subdivisions (see observations) do not show any significant histocytological responses.

Although the earlier studies indicate that

a general reduction of neurosecretory material (NS M) and significant increase in the nuclear diameter of NPO neurones occur after gonadectomy (Dixit 1970, Prasada Rao & Betole 1973) and depletion of NSM occurs afrer administration of oesterogen and androgen (Viswanathan & Sundararaj 1974b), the present study shows that only certain subdivisions respond to these experiments. Injections of steroids, or subcutaneous implants of pellets of oestradiol and testosterone are known to cause a variety of regressive changes in the ovaries such as inhibition of ovarian recrudescence, inhibition of vitellogenesis or atresia of mature oocytes (Egami 1974, Goswami & Sundararaj 1968). These results suggest that increased levels of sex steroids suppress the gonadotropic secretion probably acting at the level of pituitary and/or hypothalamus due to negative feedback action. On the contrary, ovariectomy results in stimulatory changes in the gonadotrops of the pituitary (Prasada Rao et al. 1972) and in some subdivisions (NPO-P pm, NPO-P pl and NPO-P ppd) of the NPO perhaps due to reduced levels of sex steroids. It is known that in birds subjected to long days, changes occur only in some subdivisions of the paraventricular nucleus (Uemura & Kobayashi 1963).

Since many studies indicate that nucleus lateralis tuberis (NLT) undergoes changes in response to gonadectomy or variations in gonadal steroid levels (Dixit 1970, Peter 1970, Zambrano 1971, Prasada Rao & Betole 1973, Viswanathan & Sundararaj 1974a, Billard & Peter 1977) it is evident that this nucleus plays a role in gonadotropin control. The NPO may have a conjoint action with the NLT or may have a direct control on the gonadotropin secretion. Alternatively, an indirect implication of these subdivisions is also possible in the light of the recent studies on AF-negative neuroendocrine cell complexes. Although it is known that AF-positive peptidergic neurones and aminergic neurones are responsible for adenohypophysial control, the AF-negative neuronal complexes are attaining importance (Oksche 1976), with the identification of the occurrence of electron-dense granules in the AF-negative neurones of the hypothalamus which show common features with those in the AF-positive neurones, and identification of axo-sommatic and axo-dendritic synapses of the suprachiasmatic and tuberal perikarya (Oksche & Farner 1974, Oksche 1976). Further it has also been suggested that aminergic and peptidergic neurones may interact at hypothalamic level (*cf.* Knigge et al. 1972). On account of the possible existence of such synaptic contacts with AF-negative cell complexes the NPO neurones of *Clarias batrachus* may be responding to gonadectomy and EB administration.

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