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Immunocytochemical study of GnRH systems in the brain and pituitary of normal and hCG treated european eels

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SUMMARY

The distribution of GnRH was studied in the brain and pituitary of male and female normal silver eels and of males previously treated with human chorionic gonadotropin. In normal fish, GnRH perikarya were found in the olfactory bulbs, ventral telencephalon, posterior hypothalamus and rostral midbrain tegmentum. GnRH projections were observed in numerous brain territories confirming previous data based on radioimmunoassay. No striking difference could be observed in the distribution of GnRH in the brain of hCG injected males, but a spectacular increase in the number of GnRH fibers was noted in the pituitary.

INTRODUCTION

At the silver stage, the European eel, Anguilla anguilla L., is still an immature fish whose brain-pituitary-gonad axis has a low activity (1). In the male, hCG (human chorionic gonadotropin) administration stimulates gonadal development, androgen production (2) and pituitary GTH (gonadotropin) synthesis (1). Furthermore, in hCG treated males, there is a significant increase in radioimmunoassayable GnRH (gonadotropin releasing hormone) in the brain and, to a higher extent, in the pituitary (3). We report here the results gained by immunocytochemistry on the distribution of GnRH in male and female normal silver eels and in hCG treated males.

MATERIAL and METHODS

This study was conducted on freshwater female (250-350g; N=10) or male (70-100g; N=19) silver eels. The males received one injection of 250 UI hCG (Sigma) dissolved in saline solution (N=9) or saline alone (N=6), and were sacrificed 4 months after injection. The immunocytochemical technique was similar to that desribed previously (4). The specificity of the antiserum against sGnRH (salmon GnRH) has been carefully examined previously (5) and that of the immunoreaction was assessed by routine immunocytochemical controls.

RESULTS

CONTROL ANIMALS:

The overall pattern of distribution (Figure) of immunoreactive structures was similar in both normal females and saline injected males.

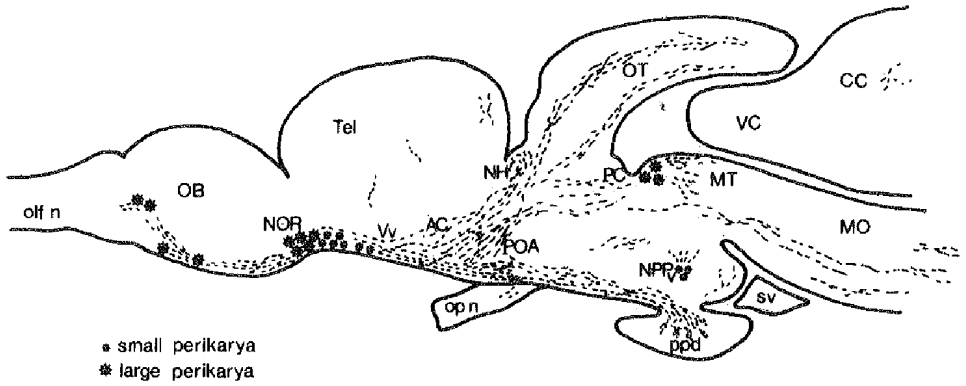

Figure: Diagram summarizing the proposed organization of the GnRH systems in the silver eel brain as seen on a longitudinal section.

AC: anterior commissure; CC: corpus of the cerebellum; MO: medulla oblongata; MT: midbrain tegmentum; NH: habenular nucleus; NOR: nucleus olfactoretinalis; NPPv: nucleus posterioris periventricularis; OB: olfactory bulbs; olfn: olfactory nerve; opn: optic nerve; OT: optic tectum; PC: posterior commissure; POA: preoptic area; ppd: proximal pars distalis; sv: saccus vasculosus; Tel: telencephalon; VC: valvula of the cerebellum; Vv: ventral telencephalon.

Immunoreactive (ir) cell bodies: The most anterior perikarya were a few bipolar cell bodies detected in the anterior part of the bulbs, adjacent to the olfactory nerves. More caudally, at the junction between the olfactory bulbs and telencephalon, a group of large perikarya was consistently found at the ventral surface of the brain. These cells form a well defined nucleus most likely equivalent to the nucleus olfactoretinalis (NOR; 6). At the level of the caudal portion of this cell group, smaller bipolar or multipolar ir perikarya were detected. This latter population extented along the ventral anterior telencephalon where they are located quite laterally. More caudally no ir cells have been detected in the preoptic area (POA) nor in any parts of the nucleus lateralis tuberis (NLT). A population of small weakly stained cells was noted in the dorsal hypothalamus at the level of the pariventricular organ. Finally, a group of very large ir perikarya was detected in the dorsal tegmentum just caudal to the posterior commissure.

Ir fibers: A continuous rostro-caudally oriented bundle was observed from the anterior olfactory bulbs to the POA. This bundle crossed all ventral areas where ir cells were described and reached the rostral preoptic region. From the POA, fibers ascended laterally along the optic tracts. A part of these fibers entered the habenular nucleus while others continued more caudally and ascended in the torus longitudinalis and the optic tectum. From the POA, an other important tract reached the pituitary stalk through the lateral preoptic region and laterobasal hypothalamus. At the level of the pituitary stalk the fibers converged to enter

the digitations of the proximal neurohypophysis where scarce ir profiles were detected. No immunoreactivity was observed in the posterior or rostral neurohypophysis nor in the adenohypophyseal cells. In addition to these tracts, numerous ir profiles were found in other brain territories such as the dorsal parts of the olfactory bulbs and telencephalon, the optic nerve, the tegmentum, the medulla oblongata.

hCG TREATED MALES:

In hCG treated males, the qualitative distribution of ir fibers and cell bodies was similar to that observed in control males or females. The most striking difference was the dramatic increase in the number and intensity of ir endings at the level of the proximal neurohypophysis in the hCG treated males compared to the controls. Although it is difficult to appreciate visually, it seemed that the density of ir brain structures was higher in hCG treated males than in controls, but this increase was not as evident than in the pituitary.

DISCUSSION

The present immunocytochemical investigation confirms that a GnRH-like factor is present in the eel brain, where it is largely distributed, in agreement with previous studies based on radioimmunoassay (7). In addition, it provides more detailed information concerning the distribution of this factor within the brain and pituitary.

The general organization of GnRH systems in the European eel is in accordance with that reported in most previous immunocytochemical studies conducted so far in teleosts (for review: 8) and more particularly in the Japanese eel (9). Indeed, the large ir cell bodies observed in the NOR of the eel have been described already in different species such as the platyfish (6,10) and the sole (11). This nucleus is a component of the terminal nerve, a cranial nerve having connections with both olfactory and visual systems and involved in the integration of environmental cues (4,12,13). The ir cell bodies along the ventral telencephalon appear similar to those previously described in the goldfish (3), according to their localisation, size and shape. The presence of ir perikarya in the periventricular diencephalic area was already reported in the stickleback (14), but in a large region extending from the dorsal hypothalamus to the dorsal thalamus. In the present study, ir neurons were detected only in a part of the monoamine containing paraventricular organ. Most likely, this area corresponds to the nucleus posterioris periventricularis of Peter and Gill (15), which was reported to contain steroid concentrating cells in goldfish (16). Large ir perikarya, similar to those observed in the eel, have already been described in the anterior dorsal tegmentum of the platyfish (6), the stickleback (14) and the goldfish (4); however, their fonctionnal significance is still unknown.

In addition to these similarities concerning the distribution of cell bodies, a high level of homology occurs in the organization of their main projections in the European eel and in others teleosts (4,6,9). Nevertheless, in contrast with most previous studies (for review: 8) no positive cell bodies could be observed in the ventral POA, which, however, contained numerous ir fibers. This absence could be related to the immature status of the silver eel. Indeed, Halpern-Sebold and Schreibman (10) have suggested that during the ontogeny of the GnRH system in the platyfish, the ir perikarya appear first in the NOR and then, at the onset of puberty, in the POA. In addition, several data suggest that the POA is mainly involved in the final steps of sexual maturation (for review: 8).

In hCG treated males, the slight elevation in brain immunoreactivity is consistent with the low increase in brain radioimmunoassayable GnRH content (3). As previously discussed (3), the effect of hCG on GnRH, as well as its effect on

GTH level (1), is probably mediated by the elevation of the production of endogenous androgens (2), although a direct effect of hCG itself cannot be, at present, totally ruled out. A positive action of sexual steroids on brain GnRH was also suggested in platyfish (17) and in trout (18). A stimulation of GnRH synthesis by sexual steroids is also documented in mammals (for review: 19). It must be pointed out that, in the hCG treated eel, only the GnRH structures already present in control animals appeared to be stimulated; in particular, the POA still did not contain any ir cell bodies as in control animals.

In the eel pituitary, the strong increase in the number and density of nerve endings in hCG treated animals is in good agreement with the finding of a large elevation (up to 10 times) in the radioimmunoassayable GnRH content (3). Furthermore, the immunocytochemical study demonstrates that GnRH is accumulated in the axonal endings of the proximal neurohypophysis; in particular GnRH does not appear to be internalized in adenohypophyseal cells as observed in the platyfish (20). This latter result strongly suggests a lack of GnRH release in the eel pituitary, a fact which could play, beside the dopaminergic inhibition of GnRH action (21), an important role in the blockade of gonadotropic function and gonadal development at the silver stage.

RESUME: Etude immunocytochimique des systèmes à GnRH dans le cerveau et l'hypophyse d'anguilles européennes normales et traitées à l'hCG.
Nous avons étudié la répartition de la GnRH dans le cerveau et l'hypophyse d'anguilles argentées normales mâles et femelles ainsi que de mâles traités à l'hCG. Chez les témoins, des péricaryons à GnRH ont été trouvés dans les bulbes olfactifs, le télencéphale ventral, l'hypothalamus postérieur et le tegmentum rostral. Des projections à GnRH ont été observées dans de nombreux territoires du cerveau, en accord avec les données des dosages radioimmunologiques. Chez les mâles traités à l'hCG, nous n'avons pas observé de modifications importantes de la répartition cérébrale de la GnRH mais nous avons noté une augmentation spectaculaire des fibres immunoréactives à GnRH dans l'hypophyse.

BIBLIOGRAPHY

1. Dufour S (1985) Sci D Thesis, Museum National d'Histoire Naturelle, Université Paris 6.
2. Khan I, Lopez E, Leloup-Hatey J Gen Comp Endocrinol, submitted for publication.
3. Dufour S, Fontaine YA, Kerdelhue B (1985) Neuropeptides 6:495-502.
4. Kah O, Breton B, Dulka JG, Nunez-Rodriguez J, Peter R, Corrigan A, Rivier JE, Vale WW (1986) Cell Tissue Res 244:327-337.
5. Breton B, Motin A, Kah O, Le Menn F, Geoffre S, Preciguox G, Chambolle P. (1984) C R Acad Sci Paris Ser III 299:383-388. Breton B, Motin A, Kah O, Le Menn F, Geoffre S, Precigoux (1986) Gen Comp Endocrinol 61:109-119.
6. Munz H, Stumpf W E, Jennes L. (1981) Brain Res 221:1-13.
7. Dufour S, Pasqualini C, Kerdelhue B, Fontaine YA (1982) Neuropeptide 3:159-171.
8. Kah O (1986) Fish Physiol Biochem 2:25-34.
9. Nozaki M (1985) in "Evolutionary biology of primitive fishes", (Foreman R. ed), Plenum Press, N.Y., 433-454.
10. Halpern-Sebold LR, Schreibman MP (1983) Cell Tissue Res 229:75-84.
11. Nunez-Rodriguez J, Kah O, Breton B, Le Menn F (1985) Experientia 41:1574-1576.
12. Demsky LS, Northcutt RG (1983) Science 202:435-437.
13. Stell WK, Walker SE, Chohan KS, Ball AK. (1984) Proc Natl Acad Sci USA 81:940-944.
14. Borg B, Goos HJTh, Terlou M (1982) Cell Tissue Res 226:695-699.
15. Peter RE, Gill VE (1975) J Comp Neurol 159:69-102.
16. Kim YS, Stumpf WE, Sar M (1978) J Comp Neurol 182:611-620.
17. Schreibman MP, Margolis-Nunno H, Halpern-Sebold LR, Goos HJTh, Perlman PW (1986) Cell Tissue Res 245:519-524.
18. Goos HJTh, De Leeuw R, Cook H, Van Oordt PGWJ (1986) Gen Comp Endocrinol 64:80-84.
19. Kalra PS (1985) J Steroid Biochem 23:725-731.
20. Margolis-Kazan H, Peute J, Schreibman MP, Halpern LR (1981) J Exp Zool 215:99-102.
21. Dufour S, Delerue-Le Belle N, Fontaine YA (1984) C R Acad Sci Paris Ser III 299:231-234.