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Gonadotropin releasing hormone (GnRH) and gonadotropin (GtH) variations around the spawning period in a wild population of roach (*Rutilus rutilus*) from Leman lake. II - The male

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Abstract

As in the female (Breton *et al.*, 1988), plasma gonadotropin levels (GtH) in male roach were always the lowest the day of aggregation on the spawning areas, eventhough they were elevated 3 days earlier. The levels increased during spawning and remained at a high level in most of the animals captured out of the visible spwaning areas, 3 weeks after the beginning of the spawning activity. During the same period the GnRH contents did not significantly vary in the hypothalamic lobes, the di-met-and myelencephalon. On the contrary there were marked variations in the pituitary gland and the telencephalon in which the GnRH levels were the highest the day of aggregation when GtH plasma levels were the lowest. In these two structures they decreased during spawning in correlation with the increase of the plasma GtH levels. These results are discussed in comparison with those obtained in the female and in relation with the possible endocrine and paracrine roles of the GnRH in these structures and mainly in the telencephalon.

Keywords : Gonadotropin, GnRH, spawning, *Rutilus rutilus*, male.

Evolution de la gonadotropine plasmatique (GtH) et des contenus en GnRH de différentes aires cérébrales autour de la période de frai dans une population de gardons (Rutilus rutilus) *du lac Leman. II - Le mâle.*

Résumé

Comme chez la femelle (Breton *et al.*, 1988), les niveaux de gonadotropine (GtH) plasmatique sont toujours les plus faibles le jour du rassemblement sur les frayères alors qu'ils étaient encore élevés 3 jours avant. Ils augmentent et sont élevés pendant le frai et le restent chez la plupart des animaux capturés hors des zones visibles de frai, 3 semaines après le début de celle-ci. Au cours de la période étudiée, les contenus en GnRH ne varient pas significativement dans l'hypothalamus, le di, le met et le myélencéphale. Par contre, on constate d'importantes modifications dans l'hypophyse et le télencéphale dans lesquels le GnRH est le plus élevé le jour de l'aggrégation quand la GtH plasmatique est au contraire la plus faible. Dans ces deux structures, ils décroissent pendant le frai corrélativement à l'augmentation des niveaux de GtH plasmatique. Ces résultats sont discutés, en comparaison avec ceux obtenus chez la femelle, par rapport aux relations GtH-GnRH et aux rôles possibles endocrines et paracrines du GnRH de ces structures et plus spécialement du téléencéphale.

Mots-clés : Gonadotropine, GnRH, frai, *Rutilus rutilus*, mâle.

INTRODUCTION

Depending upon the species, spermiation in fish may or may not be associated with an increase in the plasma gonadotropin (GtH) level. Thus, in a salmonid such as the rainbow trout, *Salmo gairdneri*, the beginning of spermiation is not correlated with the level of plasma GtH (Fostier *et al.*, 1982), GtH levels being determined after daily sampling which did not take into account the possible existence of a circadian rhythm for GtH secretion, as demonstrated in reproducing females of the same species (Zohar *et al.*, 1986). In contrast in a cyprinid such as the goldfish, *Carassius auratus* (Kyle *et al.*, 1985; Ueda *et al.*, 1985; Kobayashi *et al.*, 1986) and a castostomid taken from the wild, *Catostomus commersoni* (Stacey *et al.*, 1984), a surge of plasma GtH occurs during spawning behavior and the initiation of spermiation.

These apparent differences between species could depend on the ability of the fish to reproduce naturally. Rainbow trout kept in captivity do not reproduce and reproductive behavior seems to be necessary for gonadotropin surge at spermiation. Several factors, including environmental and social, are certainly involved in determining the GtH surge (Stacey *et al.*, 1986). Thus in a wild population of rainbow trout, a GtH surge occurred only if males were in the presence of active nest building females (Liley *et al.*, 1986). This action was probably mediated by a female pheromone, as demonstrated in the male goldfish (Kobayashi *et al.*, 1986), which may be the 17 α-hydroxy 20 β-dihydroprogestérone (Dukla *et al.*, 1987). Social, pheromonal and environmental factors are certainly integrated in the central nervous system (Demski and Hornby, 1982). Lesions or stimulations of the brain induce spermiation (Kyle and Peter, 1982). In the male as in the female, GtH secretion is under the control of GnRH, this peptide being able to stimulate spermiation in several species after GtH stimulation (Takashima *et al.*, 1983; Billard *et al.*, 1983). Thus, it is highly probable, that brain lesions, pheromonal, social and environmental factors could act to modify the neuroendocrine balance in GnRH content in order to induce the GtH surge.

Before investigating experimentally each of these factors on the GnRH equilibrium in the brain, the aim of this work was to investigate the natural brain variations of this peptide in correlation with GtH secretion. The work was done in a wild population of roach *Rutilus rutilus* and completes the results already presented for the female roach (Breton *et al.*, 1988).

This is also the first report on the quantitative variations of GnRH in a male teleost during the reproductive period.

MATERIALS AND METHODS

These have generally been described previously (Breton *et al.*, 1988). Fish were obtained from Leman lake and had the same biology and reproductive strategy as the females. After migration, they suddenly aggregated within 1 day, just preceding the beginning of spawning, so called Do in these experiments. Some weeks before spawning, fish were already giving sperm, but very few, and the spermatocrite was rather elevated (more than 90%) indicating a very weak sperm fluidification. Conditions for fish capture were the same as those described for the female. Since males were more scarce out of spawning areas, some samples were missing for the first year of the study. According to the possibilities of capture not less than 5 animals were taken at each sampling. They weighed between 150 and 200 g and were 2 or 3 years old.

After being caught, blood samples were taken and the fish killed by decapitation, pituitaries and pieces of brain were dissected and immediately deep frozen in liquid nitrogen.

The first year, brains were cut in three parts as in the female (Breton *et al.*, 1988). The following year the dissection was done as indicated on *figure* 1.

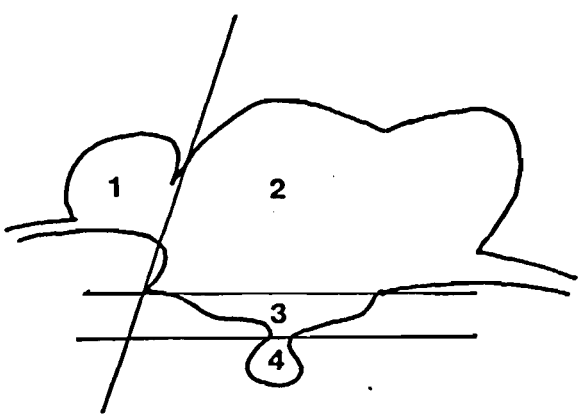

Figure 1. — Diagrammatic representation of the dissection axis in 1986.
1. teleencephalon including olfactory bulb and the preotic region;
2. di- met- and Myelencephalon except the hypothalamic lobes;
3. hypothalamic lobes;
4. pituitary gland.

After acidic extraction (Breton *et al.*, 1988), GnRH was measured in brain and pituitary glands according to Breton *et al.*, (1984 *a*). GtH was determined by an heterologous radioimmunoassay using carp GtH as the label and standard, and an antibody directed against the carp GtH β subunit (Breton *et al.*, 1984 *b*).

Results were analyzed by the Student *t*-test, variance analysis and the determination of the correlation index between GtH and GnRH.

RESULTS

GtH levels

As in the female, the pituitary GtH contents did not show any significant variations extending from 1 month before ♀ to 1 month after spawning (*fig.* 2*a* and *b*) and the amount of pituitary GtH was of the same order as that described in the female.

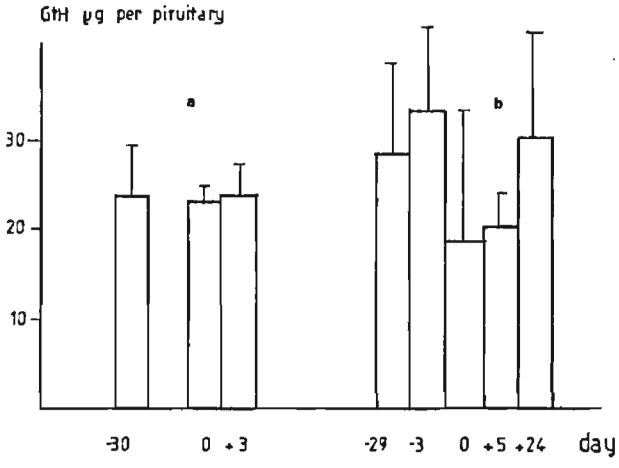

Figure 2. — Evolution of the GtH pituitary contents in male roach in 1985 (*fig.* 2*a*) and 1986 (*fig.* 2*b*) X ± SD.

Over the two consecutive years, as in the female, the plasma GtH levels were low at Do, the day of aggregation. At this time they were significantly lower than 3 days before aggregation ($p<0.01$). There was an abrupt and significant surge of GtH during spawning (*fig.* 3*a* and *b*). Contrary to the female in which plasma GtH decreased after ovulation, in the male levels were maintained high throughout the reproductive period, even in fish caught 3 weeks after the beginning of spawning out of the spawning areas (*fig.* 3*a* and *b*). At this time fish were still spermiating.

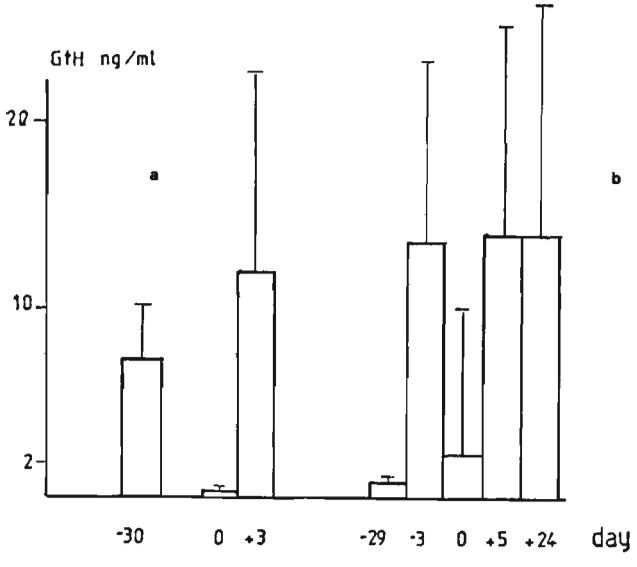

Figure 3. — Evolution of the plasma GtH levels in male roach in 1985 (*fig.* 3*a*) and 1986 (*fig.* 3*b*) X ± SD.

Before aggregation GtH plasma levels varied, being significantly higher 3 days before ($p<0.05$) and comparable to the levels measured during spawning (around 15 ng/ml).

GnRH contents

Figure 4 gives the evolution of the total GnRH in the whole brain. The GnRH levels were high when the plasma GtH was low and vice versa. They decreased during spawning when plasma GtH levels increased. These general variations have been more precisely analyzed in different brain compartments. In a first analysis the variations were not so marked as in the female (Breton *et al.*, 1988).

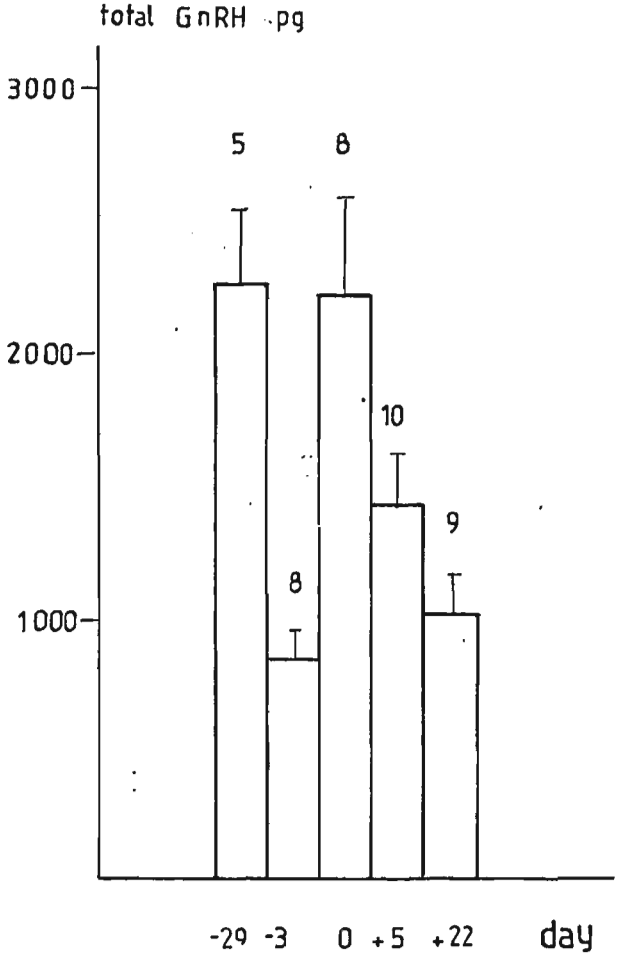

Figure 4. — Variation of the total GnRH contents in the total brain of the roach in 1986 (X ± SD).

In 1985, 30 days before aggregation, the telencephalon including the olfactory bulb and the preoptic region contained 596±227 pg of GnRH ($n=6$). This value dropped to 185±145 pg at Do ($n=5$) and remained at the same level at the beginning of spawning D1 214±9 pg ($n=9$). In the posterior brain, including the hypothalamus, on the same days, the GnRH contents were respectively: 510±138 pg (D-30), 166±77 pg (Do) and 432±117 pg (D1). Finally in the pituitary gland the lowest GnRH was measured on D-30 (58±11 pg). It was significantly higher at Do (885±254 pg) and not significantly different to that on D1 (858±271 pg).

The following year, in 1986, high GnRH levels were measured on D-30 in the telencephalon (1 127±327 pg, $n=7$) but also on Do (1 082±265 pg, $n=8$) *fig.* 5*a*. This last value corresponded to a dramatic increase of GnRH from D-3 to Do. In fish undergoing reproductive activity the telencephalon GnRH dropped significantly.

In the remaining part of the encephalon, except for the hypothalamic lobes, the GnRH contents were mainly constant and low, but decreased 1 month after spawning (*fig.* 5*b*).

In the hypothalamic lobes, the only statistical difference was a depleted level at D-3 (181±41.5 pg, $n=8$), which was not further noticeable on Do (around 500 pg). The beginning of the spawning activity was not followed by a drop in the hypothalamic GnRH (*fig.* 5*c*).

In contrast, in the pituitary, there was a drastic depletion in the GnRH content during spawning increase, being highest on Do (*fig.* 5*d*).

DISCUSSION

As in the female (Breton *et al.*, 1988), GtH plasma levels in males varied rapidly around spawning. They were lowest on the day of aggregation, being higher

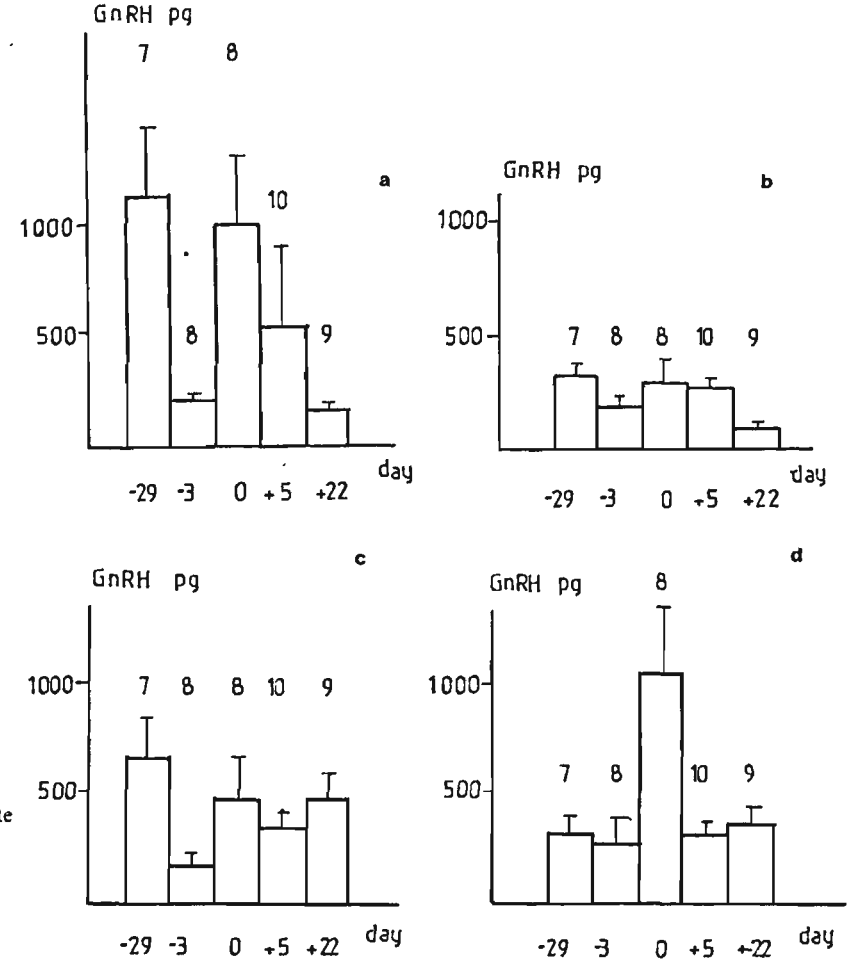

Figure 5. – Variations of the GnRH in discrete brain areas in 1986 (X±SD).
a. in the telencephalon;
b. in the di- met- and myelencephalon;
c. in the hypothalamic lobes;
d. in the pituitary gland.

3 days before and just after. Such low levels of plasma GtH have already been described during the prespawning period in the male *Catostomus commersoni* (Stacey *et al.*, 1984). In this species there was no GtH surge within the 10 days preceding the beginning of spawning, contrary to the case in both sexes in the roach. Thus this GtH rise might have a physiological significance in relation to the preparation of spawning. As far as the biology of the two species is concerned, in *Catostomus* all sampling was done in fish having already migrated from a lake to a stream in which the temperature was mainly constant and low (Stacey *et al.*, 1984); whereas in roach, fish had migrated to the shore for the day before the beginning of spawning, when the water temperature increased rapidly. Thus the rise in GtH secretion in roach might be linked to the final migration of the fish, in which the sudden rise in temperature, is probably one of the main factors triggering the spawning activity. The latter was marked by a new increase in GtH secretion. A similar increase occurred in male with the presence of ovulated and active females as in the goldfish (Kyle *et al.*, 1986) and rainbow trout (Liley *et al.*, 1986).

It was not possible to measure sperm production because fish that were net caught were sometimes stripped when released from the mesh of the net. It was also not possible to detect fish which had participated or not in spawning activity just prior to being caught. However the spermatocrite was measured before and during spawning. It was greater than 90% before and dropped to 55-25% in fish taken from the spawning areas, indicating a sperm fluidification. Since gonadotropin is known to induce testis hydratation (Clemens *et al.*, 1964; Clemens and Grant, 1964), and increase the volume of expressible milt (Billard *et al.*, 1982), spawning GtH surge most likely stimulated the spermiation, and perhaps was involved in the induction of spawning behavior. Plasma GtH remained high even in fish taken 24 days after the beginning of spawning and from the spawning grounds. This could be linked to a discrete spawning activity maintained in deep water out of the main spawning areas, rather than the stimulation of a new wave of spermatogenesis, which has been demonstrated to start again in autumn in this species and in this lake (Escaffre and Billard, 1976). Such long term elevated plasma GtH levels were not found in *Castostomus* returning to the lake (Stacey *et al.*, 1984).

Like in the female GnRH was found everywhere in the brain of the male roach and the total GnRH contents which varied between 860 pg and 2,300 pg were comparable to those found in the female (Breton *et al.*, 1988). Contrary to this sex the GnRH distribution was not exactly the same. Although the telencephalon (olfactory bulb and preoptic region) always contained the greatest amount of GnRH its relative content dropped from 70% to a maximum of 45%, when on the contrary, the relative contents of both hypothalamus and pituitary gland increased.

Within a very short period, there were two consecutive rises in GtH secretion. During the first one, GnRH levels were lowered at the same time in the telencephalon, the hypothalamic lobes, and the pituitary gland, whereas only the telencephalon and pituitary GnRH dropped during the second. This seems to indicate that the mechanisms which triggered the two GtH surges may differ. Most surprising was the fact that the hypothalamic GnRH was unaffected during the GtH surge occurring during spawning, in contrast to the female in which it dropped, in all parts of the brain-pituitary complex, in both ovulated an unovulated animals. It must also be noted that the decrease was greater in telencephalon than in the pituitary gland.

As in the female the low levels of plasma GtH measured on Do corresponded to the highest GnRH contents in every structure.

In summary, our studies in female (Breton *et al.*, 1988) and male roach are in agreement with previous reports on other species concerning the role of GtH during spawning. In addition, they clearly indicate that GtH variations also exist during the prespawning period. Although the role of such variations is still unknown, they might be involved in the final migration of the fish which may be under the influence of a synchronizer. The effect of this possible synchronizer and other external or internal factors could be at least partially mediated by the GnRH pathways in the central nervous system, and especially in the telencephalon and the hypothalamo-pituitary complex. The study of the interactions between these factors: temperature, photoperiod, spawning substrate, social environment, pheromone etc. and the GnRH neuroendocrine balance would certainly be of great importance for a better understanding of the parameters which determine fish spawning.

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