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

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Abstract

Plasma and pituitary gonadotropin (GtH) levels, and the gonadotropin releasing hormone (GnRH) in differents areas of the brain, were measured using radioimmunoassays during the prespawning and the spawning period in a wild population of roach (*Rutilus rutilus*). GtH pituitary levels did not vary. On the contrary GtH plasma levels showed variations during the prespawning period and were minimum on the day of arrival on the spawning areas, Do. During spawning they increased in ovulated females but also in unovulated animals and returned to low values, 1 month after. During this period GnRH varied inversely in all brain structures and was always negatively correlated with the blood plasma GtH levels. This suggests that all the brain GnRH would be involved in the control of the GtH secretion during spawning. This was not the case during the prespawning period, during which only hypothalamic and pituitary GnRH levels were correlated with GtH. There were also marked variations in the telencephalic GnRH extra hypothalamic GnRH. It is hypothetized that it could be a link between environmental factors and the hypothalamo-pituitary complex.

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

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. I - La femelle.*

Résumé

Les niveaux de gonadotropine (GtH) hypophysaires et plasmatiques ainsi que les contenus en "gonadotropin releasing hormone" (GnRH) de l'hypothalamus et différentes aires cérébrales ont été mesurés par dosage radioimmunologique. L'expérience s'est déroulée pendant la période précédant le frai et durant la période de frai d'une population sauvage de gardons (*Rutilus rutilus*). Les contenus hypophysaires en GtH ne varient pas, contrairement aux niveaux plasmatiques de cette hormone, dont les concentrations sont toujours les plus faibles le jour du rassemblement sur les aires de frai. Ils augmentent chez les femelles ovulées, mais aussi chez les poissons non ovulés. Pendant le frai les contenus en GnRH varient en sens inverse de la GtH dans toutes les structures cérébrales et sont corrélés négativement aux niveaux plasmatiques de GtH. On retrouve des variations similaires de la GtH et du GnRH hypophysaire et hypothalamique avant le frai. Mais, durant cette période, bien que les contenus en GnRH télencephalique montrent des variations importantes, ils ne sont pas correlés aux niveaux de GtH plasmatique. Ces résultats sont discutés et plus spécialement les rôles éventuels du GnRH extrahypothalamique. Il est fait l'hypothèse d'un rôle de médiation des facteurs de l'environnement par le GnRH télencephalique.

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

The literature concerning the evolution of the gonadotropin (GtH) plasma levels around the spawning period in fish is now well documented. In all species studied the final steps of the reproductive cycle, maturation and ovulation are associated with an increase of GtH plasma levels, as in salmonids (Breton *et al.*, 1983; Scott *et al.*, 1983) and cyprinids (Stacey *et al.*, 1983; Santos *et al.*, 1986), which generally corresponds to a circadian rhythm of GtH secretion as in the goldfish (Stacey *et al.*, 1983) and the rainbow trout (Zohar *et al.*, 1986). Most of these studies have been carried out on animals kept in captivity, in which the natural different environmental components: social, pheromonal, biotical and abiotical were not simultaneously present. There are only a few studies in which animals were sampled from the wild. This is the case for the rainbow trout (Liley *et al.*, 1985) and the whitesucker *Catostomus commersoni* (Stacey *et al.*, 1984) in which GtH changes have been shown to be sometimes the results of social interactions. The measured plasma levels of GtH constitute the results of a chain of events involving secretion, binding to the gonads, degradation etc. They do not necessarily reflect primary modification of the endocrine balance at the central level, especially that of the gonadotropin releasing hormone (GnRH), the main stimulating factor involved in the control of GtH secretion. However in the rainbow trout the pituitary GnRH contents are correlated with GtH secretion during the periovulatory period (Breton *et al.*, 1986), which is not the case for the total brain GnRH. This does not necessarily mean that GnRH does not vary in discrete brain areas, insofar as this peptide has been demonstrated in numerous parts of the central nervous system in fish either after immunocytochemical studies (Schreibman *et al.*, 1979; Kah *et al.*, 1986; Nunez-Rodriguez *et al.*, 1986) or radioimmunological measurement (Dufour *et al.*, 1982). More recently it was shown that most of the radioimmunoassayable GnRH of the brain areas varied altogether with the plasma GtH levels during the periovulatory period in the goldfish (Yu *et al.*, 1987), the work being done in isolated female kept in aquaria.

For this reason, we have attempted to analyse the brain gonadotrop axis in detail, involving the measurement of GnRH in different areas of the brain and the pituitary gland plasma gonadotropin levels, in a species taken from the wild, having a characteristic spawning behavior. Some environmental parameters have also been recorded in order to determine their possible involvement in the control of the gonadotropic activity of the brain pituitary axis. This work was carried out in both sexes. This first paper presents the results obtained in the female.

MATERIAL AND METHODS

The animals

The experiments were conducted during two consecutive years on a wild population of roach *Rutilus rutilus* taken from lake Leman. All the sampling was done at the INRA Limnological institute in Thonon-les-Bains. The roach underwent gametogenesis in autumn in the deep water of the lake, the water temperature being one of the most important parameters for the vitellogenesis complexion (Gillet, unpublished data). As the gametogenesis progressed, they started to migrate to the shore of the lake following warmer water stratification. Within 1 or 2 days, they suddenly aggregated on the shore, under the influence of an unknown synchronizer. This was probably the temperature, which must be greater than 17 to 18 °C in the spawning areas. Only the fish in active gametogenesis, or undergoing maturation migrated, others remained in the deep water and could not be captured by net catching. Thus the population was physiologically homogenous. The spawning started the day after aggregation and lasted as long as the temperature was maintained above 17°C. In these experiments, the day of arrival on the spawning ground has been determined as Do. None of the females ovulated on Do. First ovulations were detected the day after.

Samples

Fish were net caught. Nets were immersed at night and raised the following morning at 9.00 am. Fish were then rapidly unnetted and killed within the following hour. Generally 2 to 3 years old fish, weighing between 200 and 250 g, were killed at each sampling.

Blood samples were taken from all fish from a caudal vessel with a heparinized syringe. The blood was centrifuged for 15 minutes at 4000 Rpm, at 4 °C. The plasma was collected and kept frozen until it was used for GtH-RIA determination.

Fish were then killed by decapitation, and pituitaries and pieces of brain were dissected and immediately deep frozen by plunging them into liquid nitrogen. Dissections were done according to the schematic representation in *figure* 1 *a* and 1 *b*, after histological control of the brain nucleus position. The first year (*fig.* 1 *a*) GnRH was measured in the telencephalon, the remaining part of the brain (including di-, mes-, met- and myelencephalon) and in the pituitary gland. The second year (*fig.* 1 *b*), the same measurements were done, but in addition in another group of fish GnRH was measured, in the total brain, excepted the hypothalamus, the hypothalamic lobes and the pituitary gland. Ovaries were dissected for the determination of the gonadosmatic index (GSI) or the state of ovarian maturity (ovulation).

RIA determination

Pieces of brain and pituitaries were extracted by cold acetic acid (1 N), after homogenization in a glass teflon homogenizer. The homogenates were centrifuged for 30 minutes at 4°C. 50 µl of the pituitary extract was mixed with an equal volume of 0.5 M pH 7.6 phosphate buffer and left for 24 hours at room temperature to allow GtH subunits reassociation before

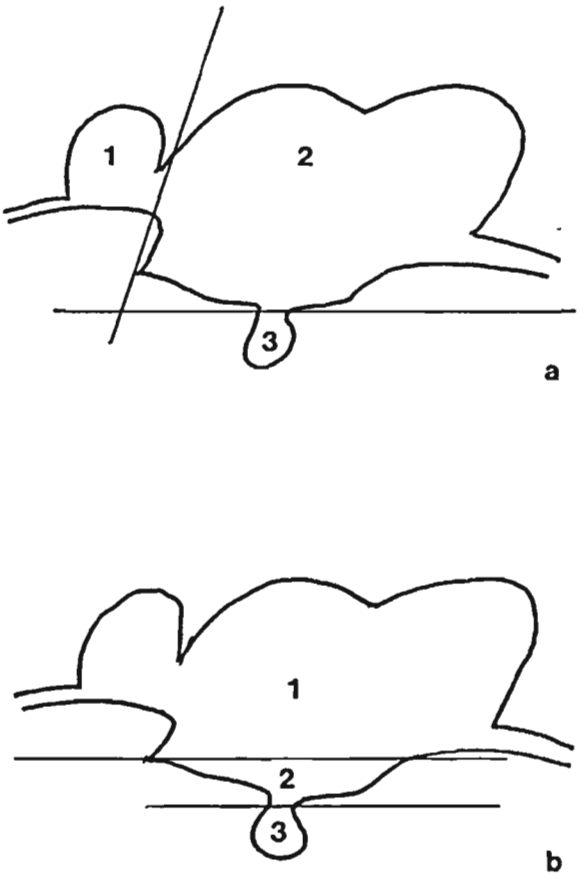

Figure 1. — Schematic representation of the dissection of the roach brain.
a. 1985 : 1 olfactory bulb and telencephalon inclunding the optic nerve and the preoptic region; 2 (remaining part of the brain including dis mes- met- and myel encephalon and hypothalamic lobes); 3 pituitary gland.
b. 1986 : 1 total brain excepted hypothalamic lobes; 2 hypothalamic lobes; 3 pituitary gland.

GtH-RIA determination. The remaining parts of the pituitary extracts and other brain extracts were heated for 10 minutes at 60°C, centrifuged again in the same conditions and preserved frozen until GnRH-RIA determination.

GtH-RIA was done using a carp GtH as label and standard, and an antibody directed against the carp GtH β-subunit (Breton *et al.*, 1984 *a*). In this system the roach gonadotropin specifically competed the carp GtH.

GnRH-RIA was performed according to the method already described (Breton *et al.*, 1984 *b*, 1985). All samples were assayed in triplicate.

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

RESULTS

The evolution of the temperature is given in *figure* 2 *a* (1985) and 2 *b* (1986). The GSI increased regularly

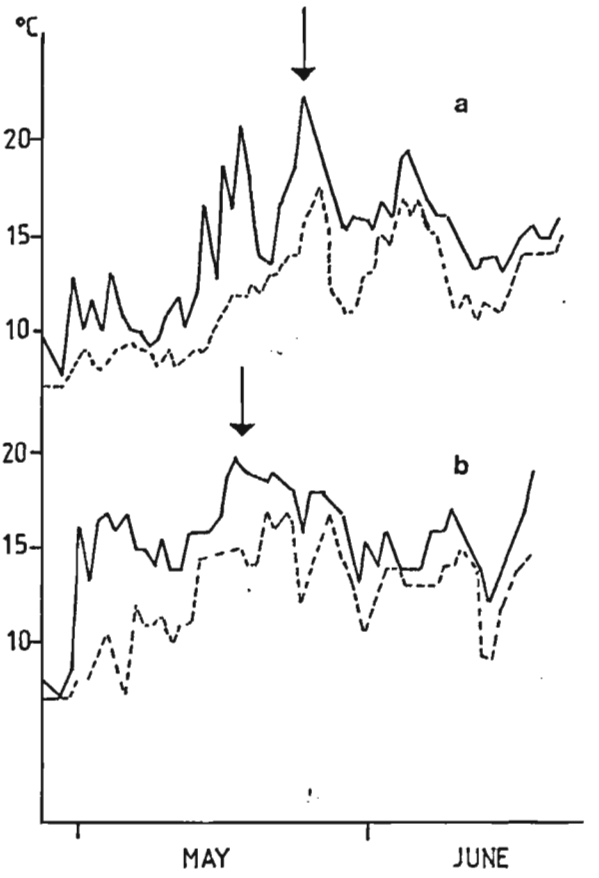

Figure 2. — Evolution of the water temperature during the 2 consecutive years *a*: 1985; *b*: 1986 (———:surface temperature; — — —: bottom temperature; →: time at which the spawning started).

from 14,32±1,82% in April to 17.66±1.84% on May 14 and finally 20.29±2.3% on May 22 at the beginning of the spawning time.

GtH levels

During the 2 years, there was no statistical differences in the pituitary GtH contents, over the period extending from 1 month before aggregation to 1 month after (*fig.* 3 *a* and *b*).

The lowest plasma GtH levels were always found on the day of fish arrival to the spawning grounds, just before the beginning of spawning (*fig.* 4 *a* and *b*). They were at the limit of the assay sensitivity, less than 0.05 ng/ml in 1986, and 0.19±0.22 ng/ml in 1985. Before aggregation GtH plasma levels varied, and were rather elevated and significantly higher 3 days before aggregation (12.85±2.54, $n=14$). The highest plasma GtH levels were measured in females

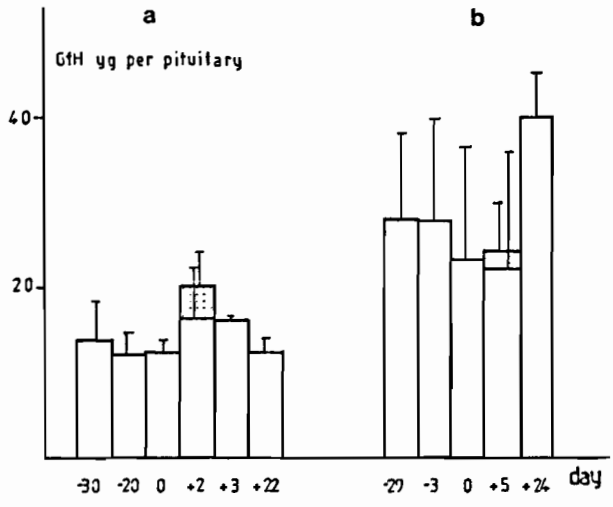

Figure 3. – Evolution of the pituitary GtH (X±SD); *a*: 1985 and *b*: 1986. (The shadowed bars represents the GtH in unovulated females).

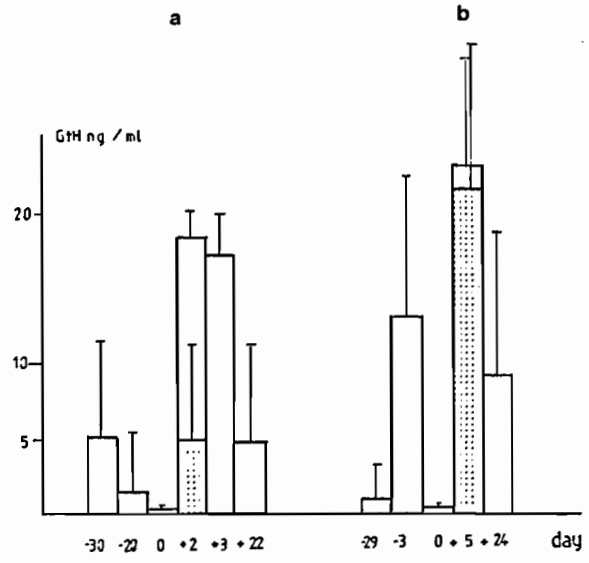

Figure 4. – Evolution of the plasma GnH levels (X±SD); *a*: 1985 and *b*: 1986. (The shadowed bars represent the GtH levels in unovulated female).

that had ovulated, but they also significantly increased in females that had not ovulated as compared to Do. In this experimental procedure, it was not possible to determine the precise time at which the animals had or would have ovulated.

Three weeks later in fish taken outside of the spawning areas, in the deep water, GtH plasma levels had decreased and returned to values comparable to those measured during vitellogenesis.

GnRH levels

In 1986, the measurement of the total GnRH content of the nervous tissue using two different methods of dissection, showed that whatever the date of sampling, GnRH contents did not statistically differ according to the type of dissection (*fig.* 5). This shows the validity of the method. Total GnRH contents were statistically at the highest on the day of arrival in the spawning areas, more than 2000 pg. They statistically decreased during spawning and reached less than 1000 pg, being statistically lower in ovulated fish than in unovulated females, in which they had already decreased as compared to values obtained on Do.

The following results have been given to try to demonstrate that these variations were more specifically linked to a definite area of the central nervous system.

In the telencephalon (*fig.* 6 *a* and *b*) G,RH contents varied before spawning. During the periovulatory period, the variations were reproducible from 1 year to another excepted on Do, at which GnRH reached the highest values measured in 1986 (1082±265 pg $n=10$) but not in 1985. The general profile was the same as in the total brain (*fig.* 5). It should be noted that in 1985, the water temperature increased to more than 20°C at which temperature the spawning would normaly have started, but a sudden drop in the temperature had arrested the phenomenon which started again some days later. In contrast in 1986 the water temperature rose gradually until the spawning began. During spawning in 1985 and 1986, the GnRH contents decreased in both ovulated (107±23 pg, $n=5$) and unovulated females (516±373 pg, $n=5$). But, because of the dispersion of the results in unovulated femals, which may indicate a less definite stage of these animals, they did not statistically differ from those measured in ovulated females. Telencephalic GnRH remained at a low level in animals outside of the spawning grounds. It correlates with the plasma GtH levels, during the periovulatory period and espacially in ovulated $r=-0.89$ and unovulated females $r=-0.82$.

In the remaining parts of the brain, including the hypothalamus, GnRH variations were quite similar from 1 year to another (*fig.* 7 *a* and *b*), and around ovulation from Do to D 24. The hypothalamic GnRH represented the main part of the GnRH from these areas. The highest concentrations were measured on Do (707±54 pg, $n=6$) in 1986. It is noteworthy that the hypothalamic GnRH increased from the vitellogenesis to Do. It dropped drastically in both ovulated and unovulated females. In these females an extra hypothalamic pool of GnRH ($p<0.01$) also remained, as well as in fish caught one month before spawning (*fig.* 7 *b*). Hypothalamic GnRH and plasma GtH levels were always negatively correlated whatever was the date of sampling.

In the pituitary gland (*fig.* 8 *a* and *b*) the profiles of GnRH contents were similar during the 2 consecutive

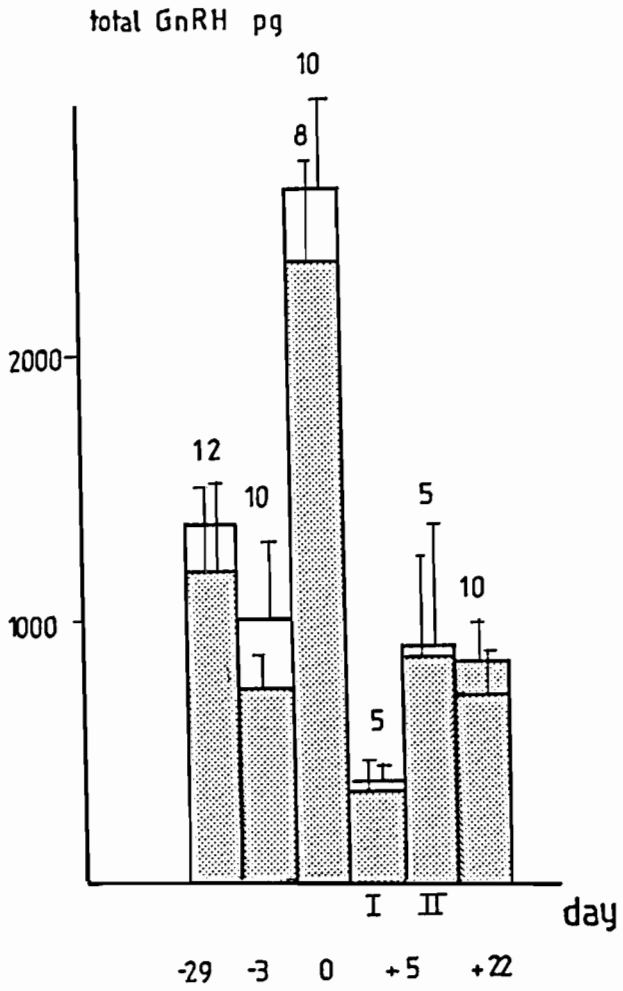

Figure 5. — Evolution of the total GnRH as measured by two different methods of dissection (X ± SE) 1986 samples.
The open bars represent the sum of the GnRH measured in the telencephalon, the remaining part of the brain and the pituitary gland.
The shadowed bars represent the sum of the GnRH measured in the brain, the hypothalamic lobes and the pituitary gland. I: in ovulated females; II: in unovulated females.

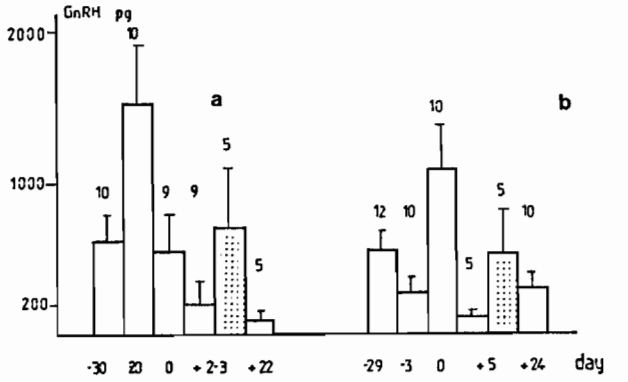

Figure 6. — Evolution of the telencephalon GnRH (X ± SE): in *a*: 1985 and *b*: 1986 (the shadowed area represents the GnRH in unovulated females).

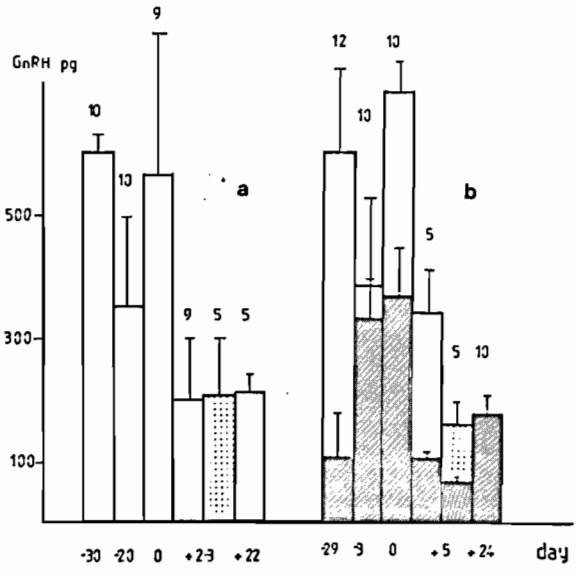

Figure 7. — Evolution of the encephalon and hypothalamic lobes GnRH (X ± SE) *a*: 1985 and *b*: 1986 (the shadowed area represents the GnRH in unvolulated females; the black bars represent the hypothalamic GnRH).

years, reaching their highest values the day of aggregation and decreasing after the beginning of the reproductive activity, in both ovulated and unovulated females in which they were depleted at the same levels.

DISCUSSION

GtH levels

These results confirm in the roach that, maturation and ovulation are accompanied by an increase of the blood plasma GtH concentrations: as in the brown trout *Salmo trutta* (Breton *et al.*, 1983) and other salmonids, the goldfish (Stacey *et al.*, 1979), the carp (Santos *et al.*, 1986) and the whitesucker *Catostomus commersoni* (Stacey *et al.*, 1984). This last species presents a spawning migration similar to that of the roach, and the lowest plasma levels were in both species measured during the prespawning period. In addition, our results demonstrated that in fact these lowest levels were obtained on the day of aggregation, levels still being high 3 days before. On Do, the low Gth levels were correlated with the highest GnRH contents in all parts of the brain. Among the various explanations, there are two possible hypothesis: either the hypothalamo-pituitary complex was at rest on that day, waiting for, or under the influence of an external synchronizer which would induce the first

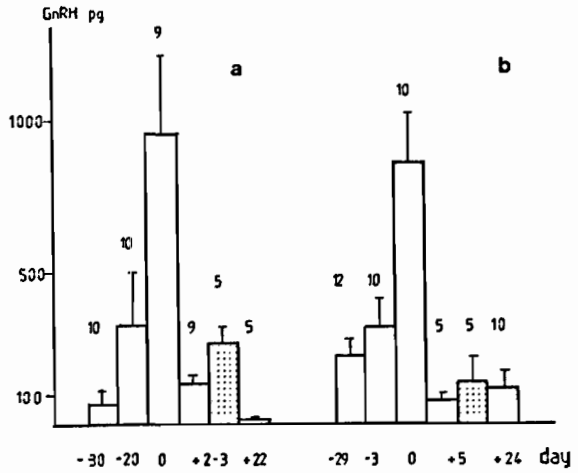

Figure 8. — Evolution of the pituitary gland GnRH (X±SE) *a*: 1985 and *b*: 1986 (the shadowed area represents the GnRH in the unovulated females).

ovulations; or this period was marked by the appearance of a circadian cycle of GtH secretion, sampling being done at a time of the day at which GtH secretion was the lowest. All our samples were collected between 9 and 10 a.m., a period of the day at which, in the goldfish Stacey *et al.* (1983) found the lowest plasma GtH level during the daily ovulatory cycle of GtH secretion. It is also during this same period that plasma GtH levels reach their minimum values during maturation in the rainbow trout (Zohar *et al.*, 1987). If this phenomenom occurred, it would be within a very short time, because, as measured in 1986, GtH plasma levels were still rather high 3 days before at the same time of the nycthemere.

During the 2 consecutive years, the plasma GtH levels of the unovulated females, taken from the spawning grounds, already increased compared with those found on Do. After stripping, these fish generally did not show any morphological feature of maturing oocytes. Thus, this GtH increase did not seem to be connected with oocyte maturation. In the female rainbow trout taken from the spawning areas, GtH plasma levels were low in green females, but high in both ovulated and nest building animals (Liley *et al.*, 1986). Thus in unovulated roach, the increasing plasma GtH levels might be the reflection of the appearance of a spawning behavior, but this phenomenon cannot be detected in individual animals, since such fish spawning in shoals and were net caught.

GnRH levels

These results confirm the wide distribution of GnRH in the brain of the roach as already demonstrated in another cyprinid the goldfish (Kah *et al.*, 1986; Yu *et al.*, 1987). The total GnRH found in this study were also comparable to those found in the goldfish (Yu *et al.*, 1987). As in this species the main pool of GnRH was located into the encephalon which generally contained about 70% of the total GnRH, and more especially into the telencephalon and the olfactory bulb. This is in agreement with the fact that the most numerous number of GnRH neurosecretory cells had been found in theses regions (Kah *et al.*, 1986). The pituitary and the hypophalamic areas contained 10 to 20% of the total GnRH. These values are comparable to those we found in the brown trout (Breton *et al.*, 1986) but higher than in the goldfish in which the pituitary GnRH represented only 5% of the total GnRH (Yu *et al.*, 1987). It must be noticed that on Do, the pituitary GnRH increased to 40% of the total GnRH as well as the olfactory bulb and telencephalon GnRH. They both return to their former values during spawning. This would indicate a very sensitive transitory endocrine change just preceding the beginning of the spawning time. This transistory situation would correspond to a period of active GnRH synthesis as far as total GnRH increased to more that 2000 pg when it was around 1000 to 1300 pg during the prespawning period. This period was followed by a rapid decrease of the GnRH in all parts of the central nervous system correlated with the increase of the plasma GtH levels. Thus all the GnRH seemed to be implicated in the regulation of GtH secretion in view of the induction of maturation and ovulation, but not with the same timing. Indeed, in both ovulated and unovulated females, hypothalamic and pituitary GnRH were depleted, in addition there always remained an unused pool of extra hypothalamic GnRH which was mobilized for ovulation. In unovulated females there were certainly several further occurring events and among these: the acquisition of a spawning behavior oocyte maturation and egg laying. This approach although it gave new information on the neuroendocrine equilibrium within the hypothalamo-pituitary complex, did not differentiate between these intricate phenomena involving also a spawning migration and a preparatory period. However, it clearly demonstrated that GnRH was detectable in all the brain structures, and is certainly involved, may be at different levels and with different modes of action, in the control of the complex mechanisms which triggered the reproductive strategy of this species.

The most striking result was the fact that the highest GnRH contents were always found in all parts of the brain on the day of aggregation, with the exception of the telencephalon (which include the preoptic area and olfactory bulb) of fish caught in 1985. This year (*fig.* 2 *a* and *b*), the water temperature first reached a threshold at which spawning would have occurred. Then, it dramatically dropped and rose again, up to 20°C. In contrast, the year after, it regularly increased. It could be supposed that this part of the brain might be one of the first concerned with temperature mediation, espacially regarding the GnRH regulation. The telencephalon was also the only part in which GnRH variations were not reproducible from 1 year to another, as was the fish

environment. This reinforces the idea of a telencephalon role in the mediation of the environmental cues variations for the modulation of the gonadotropic activity of the brain pituitary complex.

Further studies should try to analyse more precisely the neuroendocrine equilibrium during each step in correlation with the environmental, social and pheromonal components which might influence the spawning activity.

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