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REVIEW

Growth Hormone and Prolactin in Amphibian Reproduction

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INTRODUCTION

A knowledge of the natural history and life cycle of a species, and in particular its reproductive endocrinology, is most important for understanding reproduction and adaptation. As far as amphibians are concerned, it is essential for the future management of natural habitats and the perpetuation of natural populations; in addition, studies of the reproductive endocrinology of amphibians can lead to the development of models for application to studies of other vertebrates, including mammals.

Reproduction in amphibians has been well investigated with a view to understanding the endocrine and/or environmental control of seasonal reproductive cycles, and the interrelationships between the endocrine glands and their targets, and hormonal messengers. In this context, great emphasis has been placed on clarifying the role played by the pituitary hormones, such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [11, 33], while recently, the involvement of hypophyseal growth hormone (GH) and prolactin (PRL) has been elucidated.

This review focuses mainly on the involvement of PRL in courtship behavior in urodeles, and describes the effects of PRL on the development of organs related to reproduction. The relationships between plasma GH and PRL levels and seasonal changes in the reproductive organs and sex steroids in both anurans and urodeles are also described. Lastly, the direct role played by GH and PRL in inducing hepatic vitellogenin (VTG) synthesis is extensively discussed.

AMPHIBIAN GH AND PRL

Amphibian GH was first isolated from the bullfrog (*Rana catesbeiana*) pituitary glands and characterized by Kobayashi *et al.* [30]. Its amino acid sequence was determined by direct protein analysis [31] or deduced from its cDNA [54, 62] (Fig 1a). Recently, *Xenopus* GH was isolated and characterized [86], and the presence of two forms of GH, that had been previously suggested by analysis of GH cDNA [38] was confirmed. Antiserum against amphibian GH was produced using bullfrog GH as antigen, and then used to identify GH cells in various anuran pituitaries [21, 28, 53, 63, 84, 88]. However, it does not stain urodele GH cells [53]. In general, GH cells are localized in the rostral portion of the pituitary.

Amphibian PRL was first purified from bullfrog pituitaries and characterized by Yamamoto and Kikuyama [77]. Subsequently its amino acid sequence was determined by direct protein analysis [87] or deduced from its cDNA [61] (Fig. 1b). The primary structure of bullfrog PRL closely resembles that of other tetrapods. PRLs of other amphibian species such as *Bufo japonicus* [80], *Cynops pyrrhogaster* [41] and *Xenopus laevis* [85] have also been purified and characterized (Table 1). Interestingly, *X. laevis* has two forms of PRL of which the N-terminal end is free leucine, whereas other amphibian species have a single form of which the N-terminal end is blocked. All of these preparations exhibit considerable bioactivity [3, 39, 41, 77, 85]. Using these PRLs, investigators produced antisera, and the specific antisera were used for immunohistochemical studies of PRL cells in various amphibian species [1, 16, 21, 42, 52, 78, 81, 83, 84]. It is interesting to note that bullfrog PRL cells contain both immunoreactive PRL and the α-subunit of glycoprotein hormones within the same granules [63], and that in the pituitaries of larvae at early stages of development there are a few cells that are stainable with antisera against both bullfrog PRL and GH [23]. It has been demonstrated that free α-subunit is released from PRL cells and that it acts as an autocrine and/or paracrine factor to enhance the release of PRL [50]. PRL cells are quite evenly distributed in the pituitary and are often situated close to gonadotropic cells. *In vitro* studies have revealed that the responsiveness of dispersed LH cells to LH-releasing hormone is enhanced by the addition of PRL to the incubation medium, suggesting that PRL acts on gonadotrophs as a paracrine factor [51].

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AATCAATGGCTTCAGGGTTAGGCTCATCTCTTGTACTCTTGGTTGTAATATGTCTTCAAAGCCCGCAAGGGTTCAATGCCTTTCCACAGATGTCTCTTTCCAACCTCTTTACAAATGCT
                                                                                F  P  Q  M  S  L  S  N  L  F  T  N  A
                                                                               +1                          +10
GTCATTCGAGCTCAGCACCTTCACCAGATGGTTGCCGATACCTACAGAGACTATGAAAGAACATATATACCTGAAGATCAGAGATTTTCAAACAAACACTCTTATTCAGTGTATTGCTAC
V  I  R  A  Q  H  L  H  Q  M  V  A  D  T  Y  R  D  Y  E  R  T  Y  I  P  E  D  Q  R  F  S  N  K  H  S  Y  S  V  Y  C  Y
            +20                         +30                         +40                         +50
TCAGAAACCATCCCAGCACCAACAGACAAAGACAATACTCACCAGAAATCAGATATAGACCTCCTCCGCTTCTCCCTCACACTCCTGCAGTCATGGATGACCCCTATCCAAATCGTCAAC
S  E  T  I  P  A  P  T  D  K  D  N  T  H  Q  K  S  D  I  D  L  L  R  F  S  L  T  L  L  Q  S  W  M  T  P  I  Q  I  V  N
            +60                         +70                         +80                         +90
AGAGTTTTTGGCAACAACCAGGTGTTTGGAAACATTGACAGAGTTTATGACAGACTGAGGGACCTCGATGAAGGACTACACATTCTCATAAGAGAGCTGGATGATGGAAATGTTCGGAAC
R  V  F  G  N  N  Q  V  F  G  N  I  D  R  V  Y  D  R  L  R  D  L  D  E  G  L  H  I  L  I  R  E  L  D  D  G  N  V  R  N
            +100                        +110                        +120                        +130
TACGGTGTGCTGACTTTTACATATGATAAGTTTGATGTCAATCTGCGCAGTGAAGAAGGCCGGGCGAAGAATTACGGTCTACTCTCCTGCTTCAAGAAAGACATGCATAAAGTAGAGACC
Y  G  V  L  T  F  T  Y  D  K  F  D  V  N  L  R  S  E  E  G  R  A  K  N  Y  G  L  L  S  C  F  K  K  D  M  H  K  V  E  T
            +140                        +150                        +160                        +170
TACCTGAAGGTGATGAAATGCCGGAGATTTGTGGAGAGCAACTGCACCTTTTAAT
Y  L  K  V  M  K  C  R  R  F  V  E  S  N  C  T  F  end
            +180                        +190
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b

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TTACTAGCAGGAATTGGGGTGACATCACAACCAATATGCCCAAATGGGGGCACAAACTGTCAAATACCCACGTCTGCTCTCTTTGACCGTGCCGTAAAACTTTCACATTATATCCATTCT
                        Q  P  I  C  P  N  G  G  T  N  C  Q  I  P  T  S  A  L  F  D  R  A  V  K  L  S  H  Y  I  H  S
                       +1                          +10                         +20                         +30
CTTTCCTCTGAAATGTTTAATGAGTTTGATGAGCGCTTTACTCCAGGACGTAGGTTTCTAGCAAAATCTGGAATAAGTTGCCATACTTCTTCTCTGAATACTCCAGAAGACAAAGAGCAA
L  S  S  E  M  F  N  E  F  D  E  R  F  T  P  G  R  R  F  L  A  K  S  G  I  S  C  H  T  S  S  L  N  T  P  E  D  K  E  Q
                      +40                         +50                         +60                         +70
GCCCGACAGATACAACATGAAGATCTGCTGAACTTGGTGCTGAAGGTTCTAAGATCATGGAATGATCCTTTGGTTCACATGGTTTCTGAAGTGCAAGATATCCGGGAGGCTCCAGACACC
A  R  Q  I  Q  H  E  D  L  L  N  L  V  L  K  V  L  R  S  W  N  D  P  L  V  H  M  V  S  E  V  Q  D  I  R  E  A  P  D  T
                      +80                         +90                         +100                        +110
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                      +120                        +130                        +140                        +150
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G  P  A  S  I  P  G  D  E  N  S  R  L  F  A  F  Y  N  L  L  H  C  L  R  R  D  S  H  K  I  D  N  Y  L  K  L  L  K  C  R
                      +160                        +170                        +180                        +190
CTTATCCATGAGGGAAATTGTTGAACTTTTATTCTTAACTCTCTACAGCAATAAAGATGTGTTTCTCAAACAGCAATGATATGAATAAGTCTTTTAGAAAAACATGTGACAAAATCGTTT
L  I  H  E  G  N  C  end
            +198
TTTAATAGTTATATAGAGTTTCTAATAAATTGGATTCCTATTTGTAGAATAAAGCCAAATCAAATAAAATGCAAAAATATATGTACATACAAAAAAAAAAAAAAAAAAAA
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FIG. 1. The nucleotide sequences of bullfrog GH cDNA (a) and PRL cDNA (b). The predicted amino acids are shown below the nucleotide sequences. The N-terminal amino acid of the mature protein is numbered +1. Modified from refs. [61, 62]

TABLE 1. Physico-chemical characteristics of amphibian GHs and PRLs

Species		Molecular mass (kD)	pI	N-terminal	References
GH	*Rana catesbeiana*	22	7.8	Phe	[30, 31]
	Xenopus laevis I	22	6.9	Phe	[86]
	Xenopus laevis II	21	7.2	Phe	[86]
PRL	*Rana catesbeiana*	23	5.7	pyroGlu	[77, 87]
	Bufo japonicus	23	5.5	Blocked	[80]
	Cynops pyrrhogaster	23	4.7	Blocked	[41]
	Xenopus laevis I	23	5.6	Leu	[85]
	Xenopus laevis II	23	5.3	Leu	[85]

COURTSHIP BEHAVIOR

Most of the information on the role of PRL in sexual behavior is restricted to a number of urodele species. Urodeles perform a series of reproductive displays, generally in the water [59], and PRL has been assumed to play an important role in their reproduction. It induces migration into the water, where courtship by the male and oviposition by the female take place [10], and causes morphological and functional changes in the skin for osmoregulation [3, 12, 34, 44].

In the newt, *Cynops pyrrhogaster* sexually mature males

are attracted to water which has been inhabited by female newts. However, the male shows no preference for water in which oviduct-ablated female newts have been kept, indicating that sex attractant(s) is released through or secreted by the oviduct. Likewise, sexually mature females are attracted to water in which sexually active male newts have been kept ("male water"), but not to water in which abdominal gland-ablated male newts have been kept [68]. Recently, an attempt has been made to purify this female attractant from abdominal glands of the sexually mature male. The active substance was shown to be a novel decapeptide called sodefrin [26], whose effective concentration lies between 10^{-13} and 10^{-12} M, and whose stimulus has been confirmed to be mediated through the olfactory organ [67]. When the male water was passed through an anti-sodefrin column, its female-attracting activity was reduced to one-tenth of that of male water subjected to a normal rabbit IgG column, indicating that the main female-attracting activity of male water is derived from sodefrin [82]. Immunohistochemical studies, using sodefrin antiserum, revealed that it is present in the apical portion of the epithelial cells of the abdominal gland. It is worth mentioning that sexually inert females do not respond to sodefrin, but that treatment of such females with PRL and gonadotropin (GtH) restores their responsiveness (Fig. 2) [67].

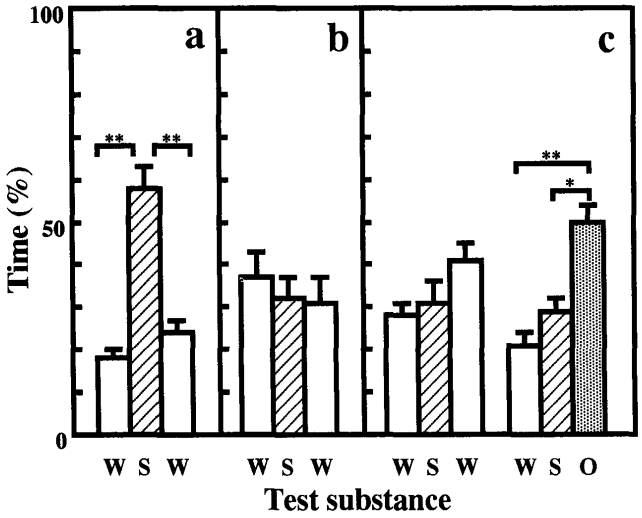

FIG. 2. Responsiveness of hormone-treated females (a), saline-injected females (b) and hormone-treated males (c) to a female-attracting peptide, sodefrin. Hormone treatment (PRL and HCG) was continued for seven days. Preference test [68] was performed 10 hr after the last injection. W, tap water; O, oviduct extract; S, sodefrin. Results represent the mean values (±SE) of eight tests performed with different test animals. *$p<0.05$ **$p<0.01$. Modified from ref. [67]

Following recognition of the sexually mature female presumably by means of the substances released through its oviduct, sexually active male newts perform courtship behavior characteristic to each species. In the newt species, *C. pyrrhogaster*, the male vibrates its tail in front of the female. During courtship the male projects fine tubules, connected to the abdominal gland, from the cloacal orifice. This is indicative of the release of sodefrin into the water to lure the female. The male then moves forward and the female follows with its snout touching the male's tail. When the male releases a spermatophore, the female picks it up with its cloaca. The effect of PRL on the initial stage of courtship behavior, tail vibration, has been studied [27, 66]. The behavior was elicited in sexually inert males by the injection of PRL of bovine, ovine, or bullfrog origin and human chorionic GtH or bullfrog LH and FSH in combination. The effects of PRL or GtH alone were less marked than those of PRL plus GtH in combination, especially in terms of frequency of the behavior. In the hypophysectomized male, administration of a combination of PRL and GtH significantly increased both the incidence and frequency of the behavior. However, PRL alone was ineffective, and the effects of GtH alone were less pronounced than they were in the intact animals receiving GtH injection, and absent in castrated animals. In the PRL-treated castrated animals, testosterone or dihydrotestosterone (DHT), but not estradiol-17β (E_2), was effective in inducing the behavior. This clearly indicates that PRL is an important hormone in the elicitation of tail vibration, and also highlights the stimulatory role of androgens. Involvement of PRL and androgens in courtship behavior has been reported for the crested newt (*Triturus cristatus*) by Malacarne *et al.* [37], who observed that its courtship activity declined after hypophysectomy and was restored by PRL replacement therapy. There is recent evidence that endogenous PRL is involved in this behavior. *C. pyrrhogaster* males, that were performing courtship behavior in the wild, were captured and injected with antiserum against newt PRL. Twenty-four hours after the treatment, both incidence and frequency of courtship behavior significantly declined, compared with a control group receiving normal rabbit serum [40]. There is, however, no direct evidence that PRL acts directly on the central nervous system involved in the manifestation of sexual behavior.

It has been assumed that androgens act centrally to elicit the sexual behavior of amphibians, mainly on the basis of autoradiographic observations that testosterone-concentrating cells, which are known to be associated with the regulation of sexual behavior in other vertebrates, exist in the brain [22]. This is also supported by the fact that the implantation of testosterone into the preoptic nucleus very effectively induces mating behavior in the frog [71]. In the newt *T. cristatus*, the courtship behavior controlling center is presumed to be located in the preoptic area, since lesions to this area abolish sexual behavior [35]. As yet, there have been no reports that GH is involved in the sexual behavior of amphibians. It should be mentioned that arginine vasotocin, but not PRL, is involved in the amplectic clasping behavior exhibited during the courtship of *Taricha granulosa* [45].

EFFECT OF PRL ON SEXUAL ACCESSORIES AND SECONDARY SEXUAL CHARACTERISTICS

PRL seems to be involved in oviducal development in the newt *C. pyrrhogaster*. Synthesis of acid mucopolysaccharides and proteins in the oviduct was markedly stimulated by PRL and E_2 in combination and to some extent to by PRL or E_2 alone. Histochemical study revealed that without PRL, sex steroids (E_2 or testosterone) could neither induce full structural development nor stimulate the synthesis of jelly substances [26] (Fig. 3). It is thus concluded that PRL is essential for oviducal jelly secretion. No definite effects of PRL on the oviducts have been demonstrated for anurans. A report that oviducal jelly secretion is elicited by PRL in *Bufo arenarum* [17] was not confirmed by investigations on *R. pipiens* and *R. catesbeiana* [2]. PRL, in addition to androgens and thyroid hormone, is implicated in the development of nuptial pads in the male red-spotted newt [60]. Nuptial color, which appears in the tail of sexually active male *Cynops pyrrhogaster*, is dependent on PRL and androgen (testosterone or DHT). PRL is also involved in cloacal gland development and secretion acting synergistically with testosterone in *C. pyrrhogaster* [24]. More recently, a similar effect of PRL on the cloacal gland of the red-spotted newt (*Notophthalmus viridescens*) has been reported [49]. It has also been reported that tail fin growth is stimulated by PRL in many Salamandridae species [25, 46, 60, 69, 70]. In the red-spotted newt, *N. viridescens* neurohypophyseal hormone blocks the action of PRL on the tail [13]. In *Cynops*, the male possesses a broader tail with a well-developed fin compared with the female. This feature of the male anatomy is suitable for vibrating the tail in front of the females during courtship. Tail height of *C. pyrrhogaster* and *C. ensicauda* was markedly increased by PRL, and the action of PRL on the tail was suppressed by E_2. The effect of androgens on the tail was not apparent [25, 27]. Therefore, development of the sexual characteristics of the tail seems to be dependent on the interaction of estrogen with PRL, at least in these two species. Singhas and Dent [60] have also observed that in male red-spotted newts kept under laboratory conditions, loss of tail height is not affected by administration of testosterone but is blocked by administration of PRL. According to Iwasawa [20], administration of testosterone to intact female *C. pyrrhogaster* a pointed tail tip causes to develop, which is characteristic of the male, so androgens may induce this structure in the male, although the author has not studied the interaction of androgens with other hormones on the growth of the tail tip.

GH AND PRL RELATED TO THE REPRODUCTIVE CYCLE

Amphibians living in temperate zones display a seasonal reproductive cycle, and their breeding success is dependent on the interplay between environmental factors and chemical signals working through the long and/or short feedback mechanisms (Fig. 4). The green frog, *R. esculenta*, living in Colfiorito pond (820 m above sea level), spends a short time in its terrestrial habitat, since it moves around for predation only in the summer. In this mountain population, the reproductive cycle is characterized by a reproductive period in late spring (May), a post-reproductive summer phase, early autumn recrudescence and winter stasis; moreover, its neuroendocrine system is extremely sensitive since, as in the bullfrog, *R. catesbeiana*, even a few minutes of captivity disables the peripheral reproductive organs through the hypothalamo-hypophysio-gonadal axis [7, 32].

The annual changes of plasma GH and PRL show marked high seasonality, related to the vitellogenic process in female frogs. Vitellogenesis in amphibians, as in all oviparous vertebrates, consists of the synthesis of the plasma egg protein precursor, vitellogenin (VTG) and its sequestration by growing oocytes, with the whole process is under multihormone control. As shown in Figure 5, plasma VTG titers have been found to change in accordance with ovarian (gonadosomatic index; GSI) changes showing two significant peaks: one at the beginning of the reproductive phase (April), and the other during the autumn recrudescence (September). The two main peaks of plasma GH that occur during the breeding period and autumn ovarian recrudescence strongly support the involvement of this hormone in the regulation of the vitellogenic process and, in turn, in ovarian growth [47, 48].

The role of GH in amphibian reproduction can be explained by a general knowledge of the well-documented somatotrophic effects of this hormone in amphibians [29], together with the fact that GH secretion is modulated by thyrotropin-releasing hormone [15]. Since vitellogenesis is without doubt a particular type of trophic process, several mechanisms controlling growth and differentiation must be taken into account, including the role of GH that has been widely recognized to be responsible for these functions.

In the male frog, GH and PRL show similar trends (Fig. 6), since the lowest levels are found between March and September; after that, GH titers rise significantly in October, as do those of PRL in December, the highest levels of GH and PRL being found during the winter months [47]. With regard to the function of PRL in anurans, in addition to the well-described evidence of Ishii *et al.* [19] and Yamamoto *et al.* [79] showing the role of this hormone in toad osmoregulation during the breeding period, these authors also found that PRL is associated with the highest plasma androgens levels and with reproductive behavior in male toads [19]. The higher winter levels of plasma PRL in both male and female *R. esculenta*, point to actions of PRL in anuran reproduction other than that previously suggested for this frog by the work of D'Istria *et al.* [14]. The seasonal changes of plasma PRL levels, and especially the increased levels of this hormone which occur at the beginning of the cold autumn recrudescence, may be speculated at least in this species, to be a response to the environmental cues which mark the end of the summer period.

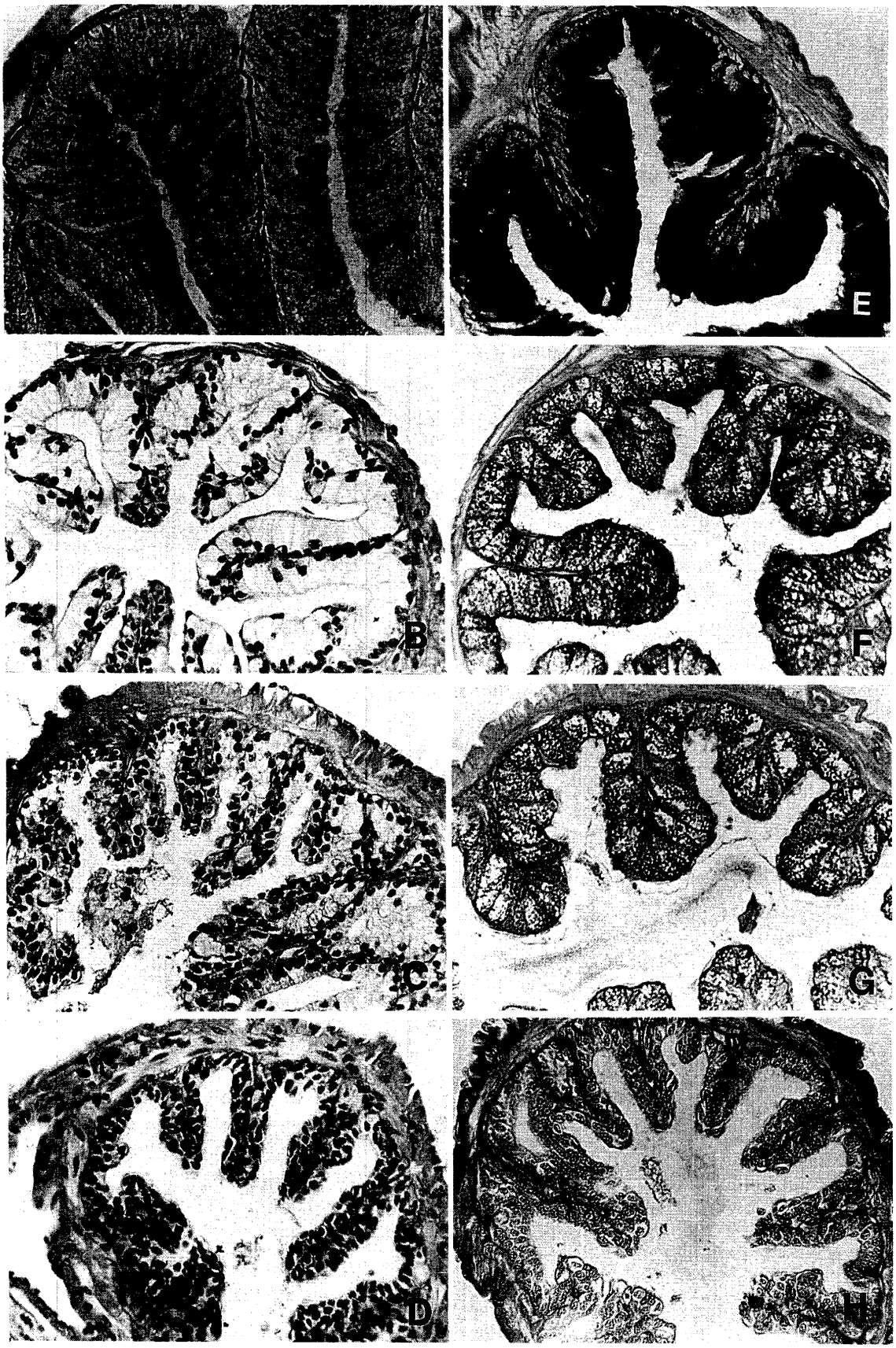

FIG. 3. Cross-section of distal portion of the oviduct of hypophysectomized and ovariectomized newts *Cynops ensicauda* treated with PRL plus testosterone propionate (A), PRL (B), testosterone propionate (C) and vehicle alone (D), and of middle portion of the oviduct of newts treated with PRL plus E2 (E), PRL (F), E2 (G) and vehicle alone (H) for 29 days (15 injections). A and H were stained with AF-azan (without oxidation), B-D with AF-hematoxylin and E-G with PAS. ×80. From ref. [25]

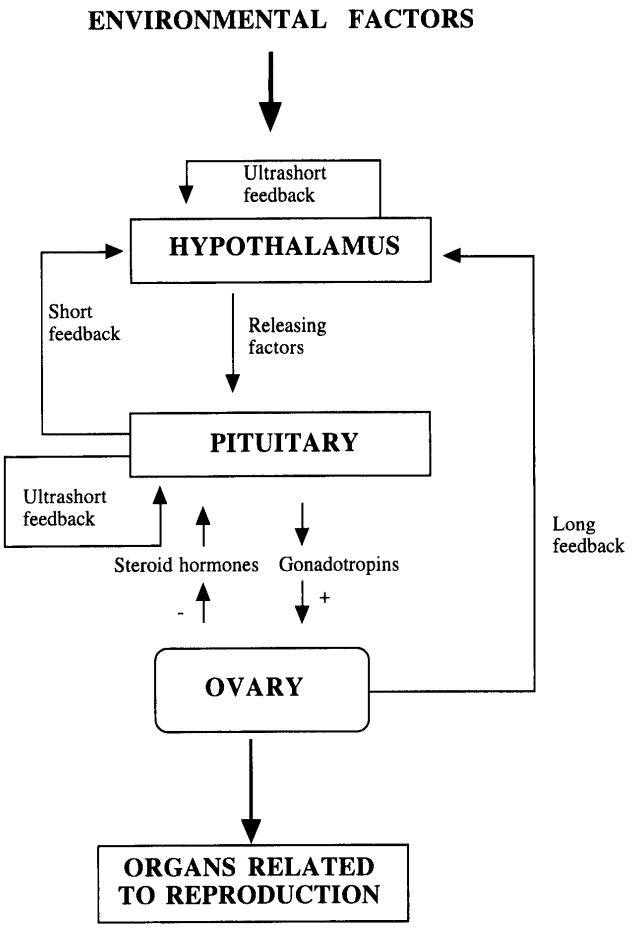

FIG. 4. The hypothalamo-hypophysio-gonadal axis regulating the seasonal reproductive cycle in amphibians together with environmental factors. Modified from ref. [7]

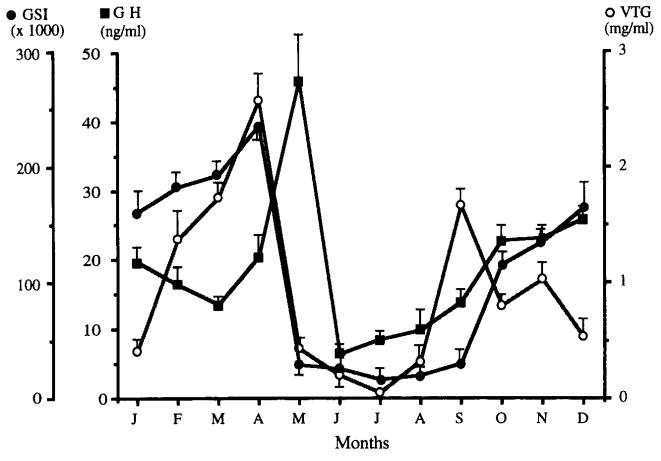

FIG. 5. Seasonal changes of gonadosomatic index (GSI), plasma VTG and GH in the female frog *Rana esculenta*. Means of 10 samples (±SE). Modified from ref. [47]

Work by Mazzi [43] and Mazzi and Vellano [44] has focused on the role of PRL in the newt, *T. carnifex*. They described cyclical changes of the organization of the hypothalamo-hypophysial system, and gonads and in water drive behavior, which is a limiting factor in reproduction. More

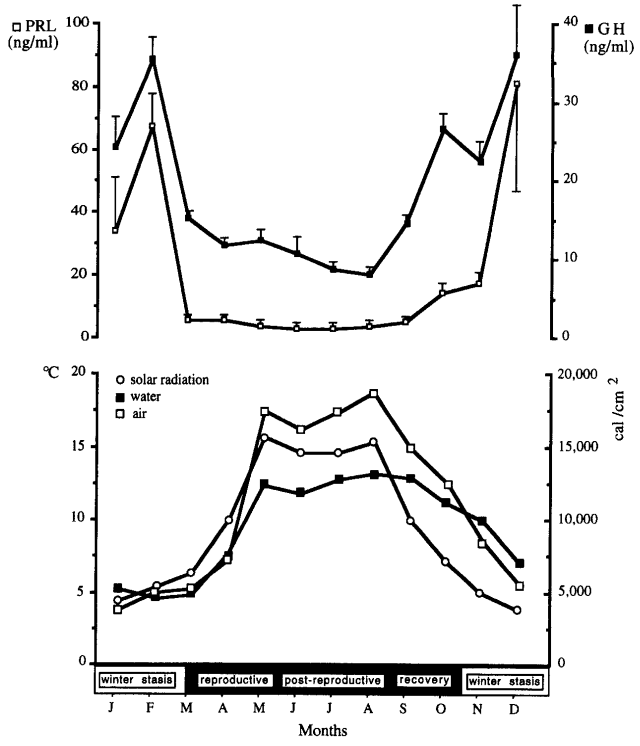

FIG. 6. Seasonal changes of plasma GH and PRL during the annual reproductive cycle of the male frog *Rana esculenta*, related to changes in water and air temperature and solar radiation during the years 1990-91. Means of 10 samples (±SE). Modified from ref. [47]

recently, the seasonal changes in plasma PRL in the wild population of *T. carnifex*. have been studied by Mosconi *et al.* [48], its annual reproductive cycle having been previously described with regard to gonadal changes and related plasma sex steroids [89]. From these studies (Fig. 7), it has been shown in male newts that plasma PRL levels, which are very low in April, increase during the winter months, in parallel with plasma androgen levels, which, as previously shown by Malacarne *et al.* [37], supports the relationship between PRL and androgen and the pivotal role they play in the reproductive behavior of the newt.

Summarizing the involvement of GH and PRL in the reproduction of amphibians, their plasma concentrations show highly seasonal trends related to cyclical changes of the reproductive organs and very marked sex differences. Moreover, PRL, together with androgens, seems to regulate sexual behavior.

GH AND PRL AND THE INDUCTION OF LIVER VTG SYNTHESIS

Our knowledge of amphibian vitellogenesis has been extensively reviewed [18, 73]. In these animals, VTG is normally synthesized only by reproductive females. Adult male animals synthesize neither the protein nor its messenger RNA. Injection of E_2 into males results in transient primary induction of VTG synthesis and its secretion into the plasma.

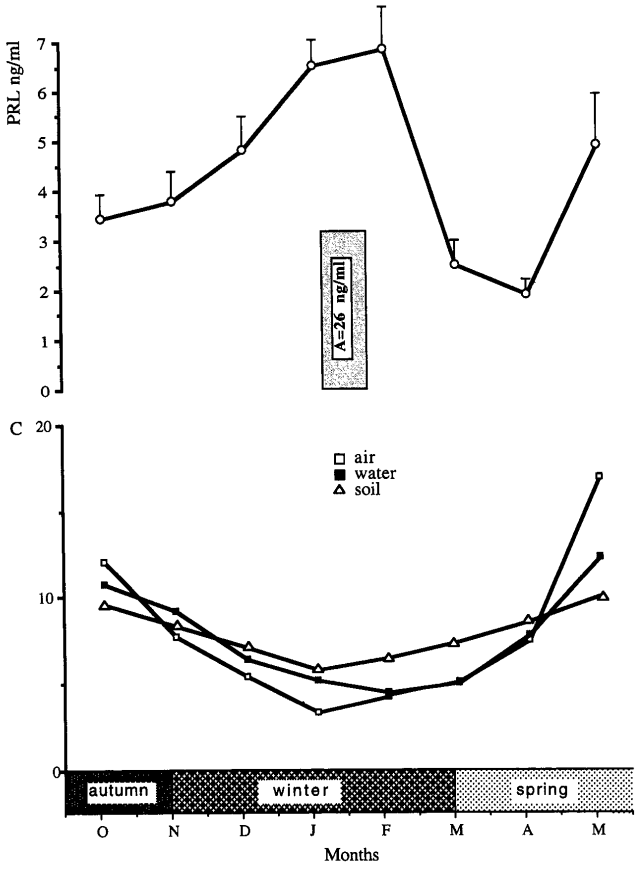

FIG. 7. Seasonal changes of plasma PRL in male newts, *Triturus carnifex Laur.*, related to ambient conditions in terms of air, water and soil temperatures during the years 1990-91; peak levels of androgens (A) occurred during the winter months. Modified from ref. [48]

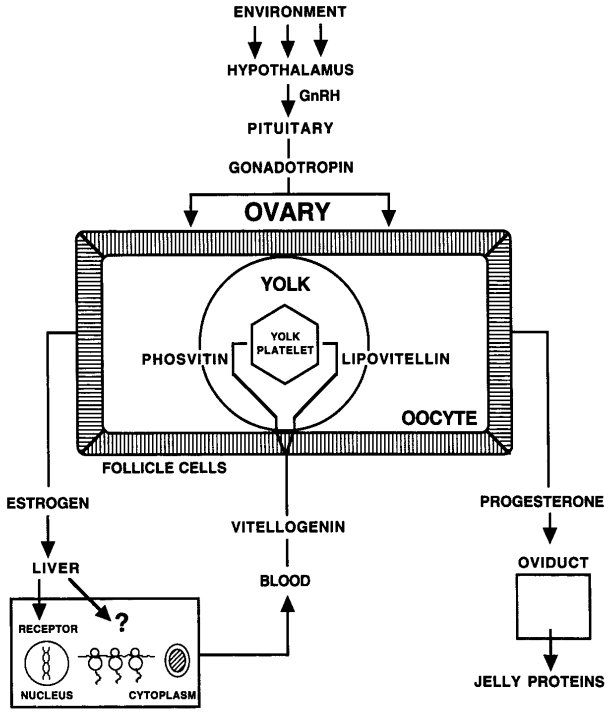

FIG. 8. Scheme summarizing the physiology of vitellogenesis in *Xenopus*. Modified from ref. [64]

In the frog, the ability of the liver to respond to estrogen stimulation is programmed developmentally; the livers of tadpoles at an early stage of metamorphosis cannot synthesize VTG, nor can they be induced to do so either *in vivo* or in culture *in vitro* [76]. VTG produced in the liver and released into the bloodstream is taken up by the ovary via receptor-mediated endocytosis, proteolitically cleaved and stored as the main yolk proteins, lipovitellin and phosvitin [74, 75]. As depicted in Figure 8, the hypothalamo-hypophysial axis is involved in the hormonal regulation of hepatic VTG synthesis, and also perhaps in its uptake by the growing oocytes. In this context, E_2 has been considered to be the hormone mainly responsible for hepatic VTG synthesis in amphibians, as in other oviparous vertebrates. Nevertheless, there is evidence of the possibility of multihormonal control of VTG synthesis and release in fish and reptiles [5, 18, 56].

In the anuran amphibian *Rana esculenta*, the injection of E_2 into both males and females can induce VTG synthesis, but the relationship between plasma estrogens and plasma VTG levels in reproductive females is of interest [57, 58]. Plasma E_2 reaches very high levels in May, concurrently with maximum VTG levels. However, highest E_2 levels occur during the summer refractory period, when VTG concentrations are basal. Moreover, in incubated liver explants from both estrogenized male and female frogs, E_2 failed to increase the very high basal levels of VTG released into the medium (Fig. 9), possibly because of desensitization of the liver estrogen receptors, whose expression has been found to vary during the reproductive cycle of this frog [55]. However, the evidence of significant amounts of circulating estrogens in amphibians which do not exhibit vitellogenesis raises questions about the role of the de-induction process at the end of the reproductive season, and about the involvement of hormones other than E_2 in amphibian vitellogenesis. In fact, Carnevali and Mosconi [6] found that during the summer refractoriness E_2 fails to induce hepatic VTG synthesis; on the contrary, homologous pituitary homogenate (HPH) can induce it in the culture medium of liver from both male and female frogs. In the same experiment conducted during the autumn ovarian recrudescence, both E_2 and HPH were able to stimulate VTG synthesis; nevertheless, that induced by HPH shows a different trend compared with that induced by E_2, and since there is no synergism between these hormones, this suggests two different types of hormonal mechanism of action in the liver (Fig. 10).

Among pituitary hormones, Carnevali *et al.* [8] demonstrated using both tissue and hepatocyte culture systems and ELISA that both ovine and bullfrog GH stimulate VTG production in cultured liver and hepatocytes from both male and female frogs in a dose-related manner, although displaying different seasonal trends (Table 2). Moreover, direct action of both ovine and bullfrog PRL has also been observed in frog liver [9] (Fig. 11). It is also of interest to note that, using Northern and dot blot hybridization, expression of VTG mRNA can be induced in frog liver stimulated *in vivo*

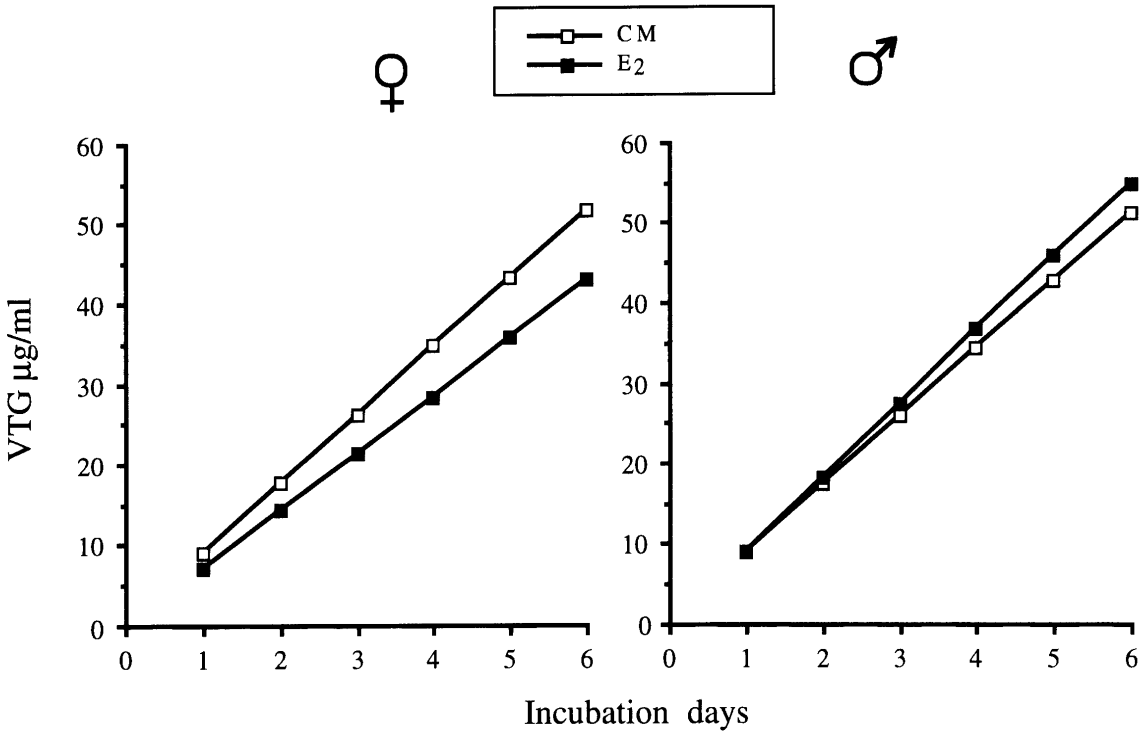

FIG. 9. VTG titers produced by *in vitro* incubation in the presence (E_2) or absence (CM) of E_2 (2 μM) of liver from both female and male frogs which had been injected with 7 μg E_2. VTG content of culture medium was assayed using an ELISA method suitable for this species, using homologous VTG antiserum. Values are expressed as cumulative amount released during the indicated period.

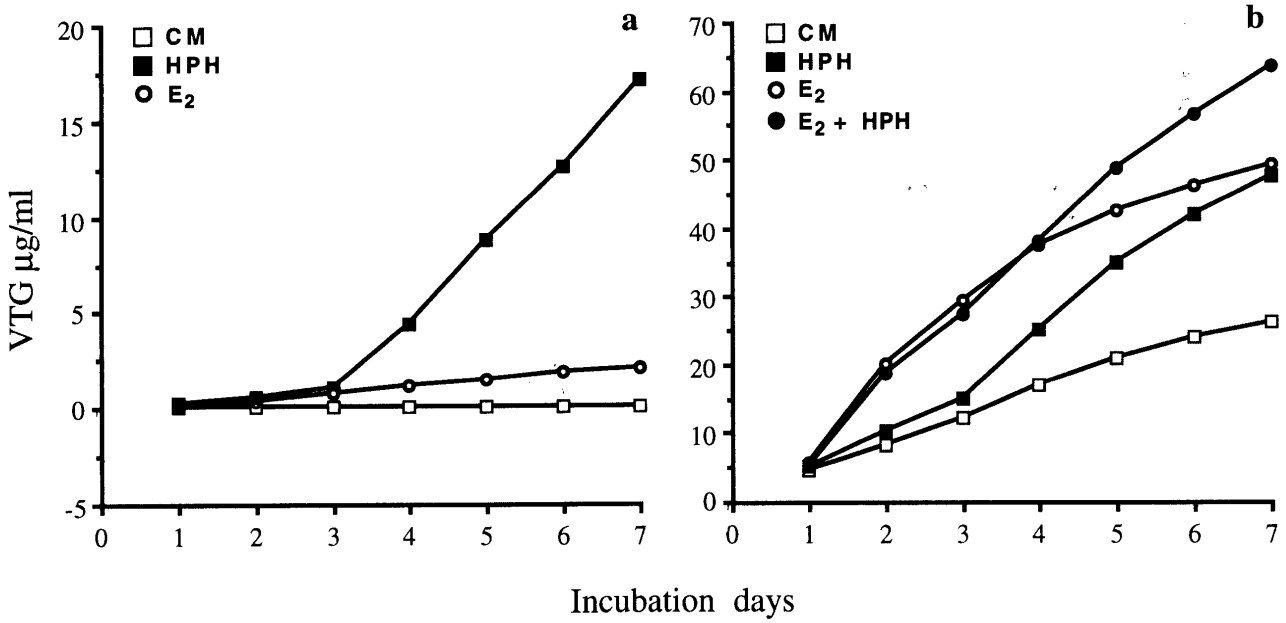

FIG. 10. VTG titers of culture medium of female frog livers measured during the post-reproductive summer phase (a) and recrudescence (b). The culture medium (CM) were supplemented with E_2 (2 μM), and/or 1/10 equivalent gland/ml of homologous pituitary homogenate (HPH). The VTG content was assayed as previously described. Values are expressed as cumulative amount released during the indicated period. Modified from ref. [6]

and *in vitro* by bullfrog GH (unpublished data). Moreover, the direct stimulatory effect of GH has also been documented in experiments carried out in both intact and hypophysectomized females (Fig. 12). VTG titers induced by GH in hypophysectomized female frogs prove much higher than found in intact females, in which hypophysectomy reduces plasma E_2 levels.

The role of the pituitary hormones in the vitellogenic process has been demonstrated in oviparous vertebrates: for instance, synergistic effects of GH in reptiles [5, 18] and the

TABLE 2. Effects of different doses of bullfrog growth hormone (fGH) in the liver culture of both female and male frogs sampled during winter stasis (January) and post-reproductive period (July). This table summarizes the direct effects of GH in inducing hepatic VTG synthesis in the culture medium, the effects being dose-dependent and related with season and sex. From ref. [8]

	TG μg/ml/day				
FEMALE					
January					
treatment					
CM	3.9±0.3	3.6±0.3	3.5±0.3	3.7±0.3	
HPH ($^1/_{10}$ eq)	4.0±0.2	4.8±0.2	6.2±0.2	6.6±0.3	
fGH					
10 ng	4.4±0.3	5.2±0.3	6.8±0.2	8.0±0.4	
100 ng	4.6±0.3	6.7±0.4	7.8±0.4	9.2±0.4	
1 μg	4.1±0.3	7.6±0.4	8.2±0.5	9.8±0.5	
10 μg	3.6±0.3	10.4±0.5	10.8±0.6	10.9±0.5	
July					
treatment					
CM	n.d.	n.d.	n.d.	0.3±0.01	0.4±0.02
HPH ($^1/_{10}$ eq)	n.d.	0.2±0.01	1.7±0.2	2.2±0.2	3.5±0.3
fGH					
10 ng	n.d.	0.4±0.01	0.4±0.02	0.9±0.1	2.4±0.2
100 ng	n.d.	0.5±0.02	1.0±0.09	2.9±0.3	3.2±0.3
1 μg	n.d.	0.3±0.01	1.5±0.1	4.0±0.3	3.7±0.3
10 μg	n.d.	0.5±0.02	2.0±0.2	4.4±0.3	4.1±0.3
——					
MALE					
January					
treatment					
CM	n.d.	n.d.	n.d.	n.d.	
HPH ($^1/_{10}$ eq)	n.d.	3.3±0.2	5.6±0.3	5.5±0.3	
fGH					
10 ng	n.d.	3.2±0.2	2.1±0.2	2.9±0.4	
100 ng	n.d.	4.1±0.2	3.2±0.2	3.8±0.2	
1 μg	n.d.	4.8±0.3	5.1±0.3	5.2±0.3	
10 μg	n.d.	5.7±0.3	6.5±0.4	6.1±0.5	
July					
treatment					
CM	n.d.	n.d.	n.d.	n.d.	n.d.
HPH ($^1/_{10}$ eq)	n.d.	0.5±0.05	1.8±0.2	2.6±0.2	4.0±0.3
fGH					
10 ng	n.d.	0.3±0.01	0.5±0.03	1.2±0.1	1.9±0.2
100 ng	n.d.	0.3±0.01	0.9±0.08	2.1±0.2	2.9±0.3
1 μg	n.d.	0.4±0.02	1.1±0.1	2.7±0.2	4.0±0.3
10 μg	n.d.	0.4±0.02	1.5±0.1	3.2±0.2	4.4±0.3
incubation days	1	2	3	4	5

CM, culture medium only; HPH, homologous pituitary homogenate; n.d., not detectable

stimulatory role played by GH in vitellogenesis in the eel [4]. The direct role played by GH and PRL in amphibian vitellogenesis gives new insight into its mechanism, whatever way these hormones work at liver level. The effects of E_2 on VTG gene expression have been well described in the work of Tata [64], Tata and Smith [65], and Whali *et al.* [72]. Indeed, other pituitary hormones can be also considered as candidates (Fig. 13).

Summarizing the effects of GH and PRL on the induction of hepatic VTG synthesis in amphibians, three main points must be considered. First, pituitary hormones other than E_2 have a direct effect on hepatic vitellogenin synthesis and secondly, both GH and PRL induce dose-dependent synthesis and release of VTG in culture media, the effects depending on season and sex. Lastly, VTG synthesis is a good model for studies of the mechanism by which steroidal

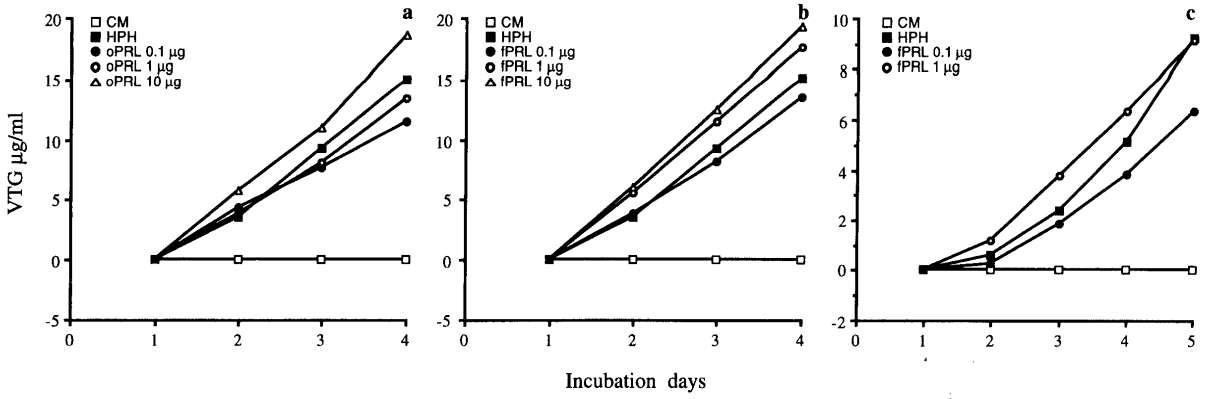

FIG. 11. VTG production in the culture medium of male frog liver. The culture medium (CM) was supplemented with different doses of ovine PRL (oPRL) or bullfrog PRL (fPRL). These experiments were carried out in January (a, b) and July (c). The direct effects of both oPRL and fPRL were found to be dose-related and dependent on the season. The values were expressed as cumulative amounts released during the indicated period. Modified from ref. [9]

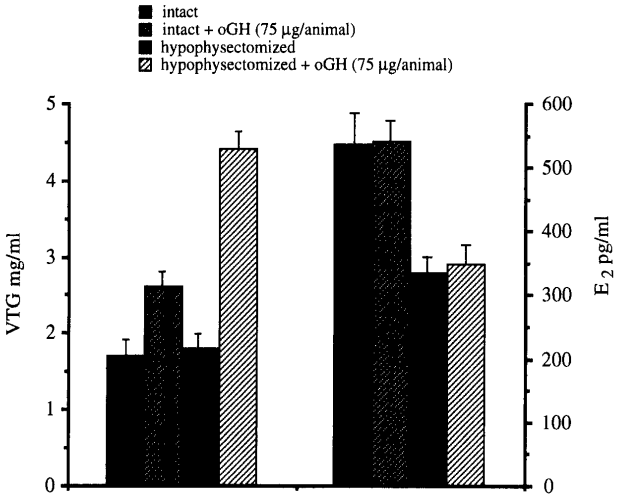

FIG. 12. *In vivo* effects of ovine GH (oGH) on the plasma VTG levels in intact and hypophysectomized female frogs. The experiments were performed during the pre-reproductive period. The values are the means of five samples (±SE). E_2 titers were evaluated by an RIA suitable for this species [54, 55].

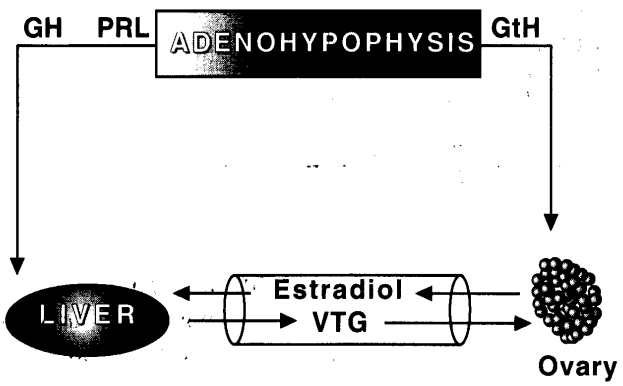

FIG. 13. The multihormonal control mechanism of hepatic VTG synthesis proposed for *Rana esculenta*.

and non-steroidal signals stimulate the same physiological responses in the same target organ.

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