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Evidence That Corticotropin-Releasing Hormone Acts as a Growth Hormone-Releasing Factor in a Primitive Teleost, the European Eel (*Anguilla anguilla*)

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Abstract

The inhibitory control of growth hormone (GH) release by somatostatin (SRIH) has been conserved throughout vertebrate evolution. In contrast, the neuropeptides involved in the stimulatory control of GH vary according to species and/or physiological situations. We investigated the direct pituitary regulation of GH release in a primitive teleost, the European eel (*Anguilla anguilla* L.) at the juvenile stage. Short-term serum-free primary cultures of dispersed pituitary cells were used, and GH release was measured by an homologous radioimmunoassay. Whereas growth hormone-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), neuropeptide Y (NPY) and cholecystokinin (CCK) failed to induce any change in GH release, corticotropin-releasing hormone (CRH) dose-dependently stimulated GH release with a significant effect at 1 nM and a maximal effect ($\geqslant$400% of controls at 24 h) at 100 nM. In agreement with our previous studies, PACAP also stimulated GH release but its maximal effect was lower than that of CRH. Proopiomelanocortin (POMC)-peptides, corticotropin (ACTH), melanotropin (α-MSH), β-endorphin) had no effect on GH release, at any dose tested (0.1–1000 nM), indicating that the stimulatory effect of CRH on GH release by somatotrophs was not mediated by CRH-induced release of POMC-peptides from corticotrophs and melanotrophs. The CRH antagonist, α-helical CRH(9–41), significantly inhibited the stimulatory effect of CRH on GH release, suggesting the implication of specific CRH receptors related to mammalian ones. The stimulatory effect of CRH on GH release was reduced after 24 h of incubation, indicating a desensitization. In contrast, no desensitization to the inhibitory effect of SRIH was observed. SRIH inhibited CRH action in a dose-dependent manner. The effect of SRIH was overriding, 1 nM SRIH being able to abolish the effect of 1000 nM CRH. In conclusion, in the eel, CRH stimulates GH release directly at the pituitary cell level. GH and cortisol secretions could interact in controlling several physiological functions such as metabolism and ion exchange. This study suggests that CRH may have played an important early role in vertebrates co-ordinating the activation of various endocrine axes involved in metamorphosis, osmoregulation, stress and fasting. The stimulatory role of CRH on GH release may have been partially conserved during evolution, as it is found in some human physio-pathological situations such as stress, fasting and depression.

In mammals, the regulation of growth hormone (GH) release is primarily mediated through the opposite actions of two hypophysiotropic factors: growth hormone-releasing hormone (GHRH) stimulates, while somatostatin (SRIH) inhibits GH release (1–4). SRIH-14 is wholly conserved among vertebrates (5). Its inhibitory action on GH release has been demonstrated in various teleost species: in the goldfish (*Carassius auratus*) *in vivo* (6) and *in vitro* (7, 8), in the rainbow trout (*Oncorhynchus mykiss*) *in vitro* (9, 10), and in the Japanese and European eels, *in vitro* (*Anguilla japonica* (11); *A. anguilla* (12)).

As to the stimulatory control of GH release, various

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neuropeptides have been shown to be active in different teleost species (13). GHRH stimulated GH release in the goldfish *in vivo* and *in vitro* (14), in the rainbow trout *in vitro* (9, 15), and in the tilapia (*Oreochromis* spp.) *in vitro* (16). Pituitary adenylate cyclase activating peptide (PACAP), which is encoded by the same gene as GHRH in teleosts (catfish, *Clarias macrocephalus* (17); salmon, *O. nerka* (18)), stimulates GH release *in vitro* in the salmon (18) and in the European eel (19). Gonadotropin-releasing hormone (GnRH) has been shown to stimulate GH release in the goldfish *in vivo* and *in vitro* (20), and *in vitro* in the tilapia (16) and in the common carp, *Cyprinus carpio* (21). A GH-releasing activity of thyrotropin-releasing hormone (TRH) has been seen in the goldfish both *in vivo* (6) and *in vitro* (22) and in the common carp *in vitro* (21). As to neuropeptide Y (NPY) and cholecystokinin (CCK), their stimulatory actions on GH release *in vitro* have been reported in the goldfish (23, 24).

This large range of GH stimulators in fish could be linked to the many roles that GH plays in various physiological functions such as growth, metabolism, reproduction, osmoregulation and immunity (3, 25). In the goldfish, a variation in the ability of the different neuropeptides to stimulate GH release was reported according to the physiological stage (13, 26). While GHRH and CCK were more potent in stimulating GH release in sexually regressed fish, GnRH, NPY and TRH had greater stimulatory effects on GH release in sexually mature fish. What is more, sex steroids influenced the responsiveness of the somatotrophs to neuroendocrine factors: GnRH-, NPY-, or TRH-induced GH release was increased by estradiol (13, 26, 27). In addition to these stage-related variations, important species-related differences in GH stimulators are also found among teleosts. GHRH stimulates GH release in the goldfish, rainbow trout and tilapia, but has a very low, dose-independent, effect in the salmon (18), and is without effect in the eel (19). GnRH stimulates GH release in the goldfish, common carp and tilapia, but not in the trout (15) and catfish (28). TRH activates GH release in the goldfish and common carp, but not in the tilapia (16).

Much of this diversity may be related to the fact that teleosts represent a phylogenetical group with a large evolutionnary diversity and a high number of species (29, 30). We therefore thought it important to investigate GH regulation in a primitive teleost, the European eel. This species belongs to the group of Elopomorphs considered to be close to the origin of teleosts, in as much this study may provide information on ancestral regulations in teleosts and vertebrates (29).

In this work, we investigated the direct effects of hypothalamic neuropeptides on GH release in the juvenile eel, using primary cultures of pituitary cells as previously described (12). Hormonal release was analysed by an homologous radioimmunoassay for eel GH (31).

Materials and methods

Animals

Juvenile (yellow stage; body weight 30–100 g) eels (*A. anguilla*) netted in ponds from the West of France were used. At the laboratory, eels were kept in running, aerated freshwater, at 12–15 °C, under natural photoperiod. Experiments were performed within one to 3 weeks after arrival to the laboratory. Animal manipulations were performed according to the recommandations of the French ethical committee and under the supervision of authorized investigators.

Peptides

Synthetic hormones: human ACTH(1–17), α-MSH, human β-endorphin, bovine CRH, human GHRH, mammalian GnRH, SRIH-14, TRH, human NPY, sulphated CCK-8, and the CRH antagonist: α-helical CRH(9–41), were purchased from Sigma (Saint-Quentin Fallavier, France). Mammalian PACAP-38 was kindly provided by Dr H. Vaudry (Laboratoire de Neuroendocrinologie Cellulaire et Moléculaire de l'Université de Rouen, Unité INSERM 413, France). Stock solutions (5.10^{-5} M) were diluted in distilled water. Further dilutions were performed in culture medium, just before addition to the wells.

Primary culture of pituitary cells

Dispersion of pituitary cells was performed using an enzymatic and mechanical procedure as previously described (32). One hundred eels were used for each experiment. Cells were cultured on poly-L-lysine (Sigma) precoated 96-well plates (Costar, Cambridge, MA, USA), at a density of 62 500 cells/well, in serum-free culture medium (Medium 199 with Earle's salt, sodium bicarbonate, 100 U/ml penicillin, 100 µg/ml streptomycin and 250 ng/ml fungizone; GIBCO, Cergy-Pontoise, France), as previously described (12). Plates were incubated in a tissue culture incubator, at 18 °C under 3% CO_2 and saturated humidity. Cells were allowed to attach and rest for 24 h before renewing the medium; the cells were then cultured for 2 more days before the start of the treatments. Replicates of six wells for controls or each treated groups were used. Media and treatments were renewed at 12 h, 24 h, 48 h and 96 h. Collected media were kept frozen (−20 °C) until GH RIA.

Radioimmunoassay

GH from culture media was assayed in duplicates using an homologous eel GH radioimmunoassay (RIA), as previously described (31). The total GH released into the culture medium was determined by cumulating GH released between each sampling time up to 96 h (12). Results were expressed as nanograms GH per 62 500 cells. Stimulation of GH release in treated wells was calculated as a percentage of mean GH release in control wells in the same experiment.

Statistical analysis

For each experiment, replicates of six wells (62 500 cells/well) were used for each treatment and values are given ± SEM. The significance of the differences between controls and treated wells was assessed by one-way ANOVA followed by Student-Newman-Keuls multiple comparison test (Instat program for Macintosh, GraphPad Software, San Diego, CA, USA).

Results

Kinetics of the effects of various neuropeptides on GH release

The total amount of GH released into the culture medium was determined by cumulating GH released between each sampling time over 96 h (12). Somatotrophs continuously released large amounts of GH during 96 h of culture in serum-free conditions (Fig. 1). This is in agreement with our previous studies (12). GHRH, GnRH and TRH (1000 nM) had no significant effect on GH release at any time of incubation, as compared to controls (Fig. 1). PACAP (1000 nM) significantly stimulated GH release ($P < 0.001$ as compared to controls, at each incubation time) (Fig. 1), in agreement with previous studies (19). CRH (1000 nM) also significantly increased GH release ($P < 0.001$ as compared to controls, at each incubation time) (Fig. 1). Furthermore, the total amount of GH released under CRH treatment was higher than that under PACAP treatment ($P < 0.01$ at 24 h; $P < 0.001$ at 48 h and 96 h). SRIH (1000 nM) strongly inhibited GH release ($P < 0.001$ as compared to controls, at each

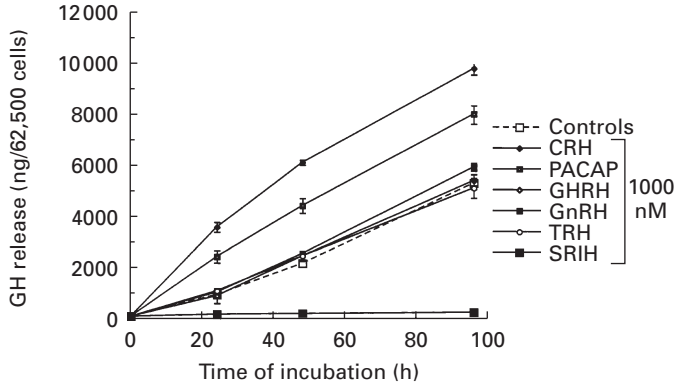

FIG. 1. Kinetics of GH release by eel pituitary cells in the absence (controls: dotted line) or presence of different secretagogues at 1000 nM. Values are given as means ± SEM (n = 6 wells; 62 500 cells/well). For CRH, PACAP and SRIH, means are significantly different ($P < 0.001$) from controls at each time of incubation (variance analysis).

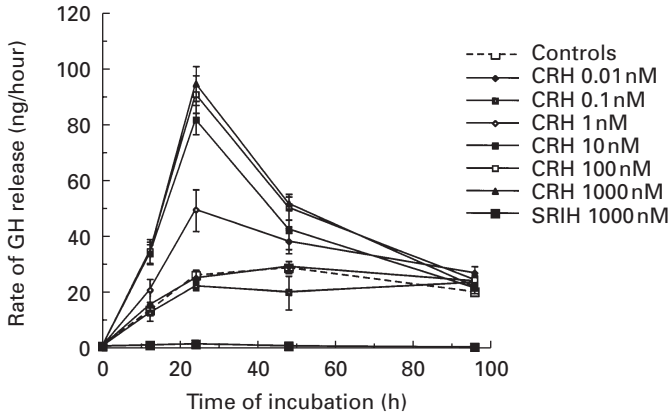

FIG. 2. Rates of GH release by eel pituitary cells in the absence (controls: dotted line) or in the presence of 1000 nM SRIH or of various doses (0.01–1000 nM) of CRH. Results are expressed as ng GH released/h. Values are given as means ± SEM (n = 6 wells; 62 500 cells/well).

incubation time) (Fig. 1), in agreement with our previous studies (12).

Rate of GH release by controls cells and by cells under CRH or SRIH treatments

Cultured pituitary cells were incubated with increasing doses (0.01–1000 nM) of CRH or with 1000 nM SRIH for 96 h. The rate of GH release (ng/h) was calculated between each sampling time. Control cells had a steady rate of GH release up to 96 h. CRH had no effect on GH release at 0.01 and 0.1 nM, but significantly increased the rate of GH release from 1 nM (49 ± 7.4 ng/h at 24 h *vs* 25.7 ± 2 ng/h in controls; $P < 0.001$) (Fig. 2). The maximal effect was at 100 nM (90.6 ± 6.6 ng/h at 24 h). After 24 h of treatment with efficient doses of CRH (1–1000 nM), the rate of GH release was significantly reduced: at 96 h, values were not different from those of control cells (for instance, 22.2 ± 0.88 ng/h for 1000 nM CRH treated cells *vs* 20.4 ± 1.7 ng/h for control cells) (Fig. 2). This desensitization to CRH seen after 24 h, contrasted with the lack of desensitization to 1000 nM SRIH. Indeed, in the presence of SRIH (1000 nM) the rate of GH release was very low and constant up to 96 h (0.62 ± 0.08 ng/h; $P < 0.001$ as compared to controls) (Fig. 2).

Dose-dependent effects of various neuropeptides on GH release

In order to further investigate the specificity of CRH action on GH release, primary cultures were incubated for 24 h in presence or absence of different concentrations of potential stimulators. CRH showed a dose-dependent stimulatory effect on GH release (Fig. 3), with a significant effect at 1 nM ($P < 0.01$), a maximal stimulatory effect (400% of controls) at 100 nM and an ED_{50} of 2.5 nM. In contrast, GHRH, TRH, NPY and CCK had no significant effect at any concentration tested (Fig. 3).

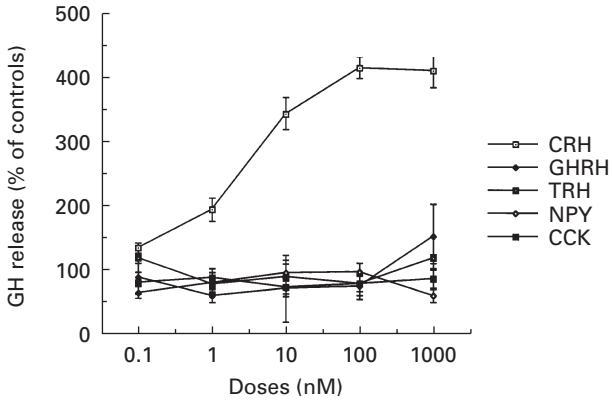

FIG. 3. Dose-dependent effects of CRH and other neuropeptides (GHRH, TRH, NPY, CCK) on GH release by eel pituitary cells. Cells were treated for 24 h. Results are expressed as percentage of GH released by control cells. Values are given as means ± SEM (n = 6 wells; 62 500 cells/well).

Effect of a CRH antagonist, α-helical CRH (9–41) on GH release

This experiment was conducted to examine whether CRH-dependent GH release in the eel could be blocked by an antagonist known to inhibit CRH action in the rat (33) and in the goldfish (34). Cells were incubated for 24 h with various doses of CRH with or without 1000 nM α-helical CRH(9–41). CRH alone caused a significant dose-dependent increase in GH release above control levels at concentrations from 1 to 1000 nM, whereas the antagonist (1000 nM) alone had no effect (Fig. 4). The CRH antagonist (1000 nM) significantly reduced the effect of 1, 10 and 100 nM CRH ($P < 0.001$ for 1 and 10 nM and $P < 0.01$ for 100 nM as compared to the same doses of CRH alone) (Fig. 4). No significant effect could be seen against the highest dose of CRH tested (1000 nM).

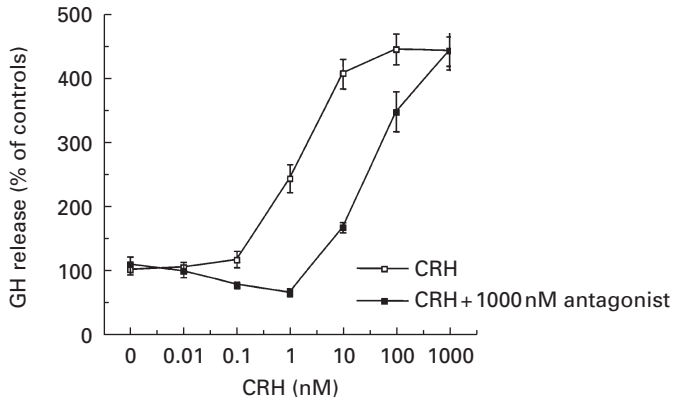

FIG. 4. Effect of CRH antagonist, α-helical CRH(9–41), on dose-dependent effect of CRH on GH release by eel pituitary cells. Cells were treated for 24 h with various doses (0.01–1000 nM) of CRH in presence or not of 1000 nM CRH antagonist. Results are expressed as percentage of GH released by control cells. Values are given as means ± SEM (n = 6 wells; 62 500 cells/well).

Dose-dependent effects of CRH and POMC peptides on GH release

To address the question of whether the stimulatory effect of CRH on GH could be mediated through its stimulatory effect on POMC peptides, we compared the effects of CRH and several POMC peptides on GH release. Various doses (0.1–1000 nM) of CRH, ACTH, α-MSH and β-endorphin were tested over 24 h. Whereas CRH showed a dose-dependent stimulatory effect on GH release, none of the POMC peptides modified GH release at any dose tested (Fig. 5).

Dose-dependent inhibitory effect of SRIH on CRH-stimulated GH release

The effects of various doses of SRIH (0.01–1000 nM) on the stimulatory action of 1000 nM CRH were tested over 24 h (Fig. 6). GH release was stimulated by 1000 nM CRH (>400% of controls; $P < 0.001$). Low doses (0.01 and 0.1 nM) of SRIH had no significant effect on CRH-stimulated GH release. At 1 nM, SRIH strongly inhibited CRH-stimulated GH release, which reached a value not significantly different from controls. At 10–1000 nM, the inhibitory effect of SRIH on GH release was stronger than the stimulatory action of 1000 nM CRH, the values being significantly lower than in controls ($P < 0.001$).

Discussion

Our study demonstrates that CRH is able to significantly stimulate GH release by eel pituitary cells. The effect of CRH is dose-dependent, with a significant effect at 1 nM and a maximal effect (⩾400% of controls at 24 h) at 100 nM. To our knowledge, the effect of CRH on GH release has not been investigated in other teleosts, thus this is the first demonstration of such a growth hormone-releasing activity for CRH in fish. This result is in agreement with the fact that in teleosts, some immunoreactive CRH fibres are localized close to the GH-cells (sea bass, *Dicentrarchus labrax* (35)).

The maximal stimulatory effect of CRH was significantly higher than that of PACAP, which has been previously reported to stimulate GH release in teleosts (salmon (18); eel (19)). Moreover, the fact that stimulators of GH release in other teleost species, such as GHRH, GnRH, TRH, NPY and CCK, had no effect in the same culture conditions, underlines the potential importance of CRH stimulatory action in the eel.

In our experiments, we used mammalian peptides as, although none of them have been yet isolated in the eel, many of them show a high structural conservation during vertebrate evolution. Indeed, identities between mammalian and teleost molecules were reported to be 95% for CRH (white sucker, *Catostomus commersoni* (36)), 92% for PACAP (salmon (37)) and 93% for NPY (*Torpedo marmorata* (38)). As to CRH action, ovine CRH was previously shown to stimulate ACTH release *in vitro* in the goldfish (39) and in

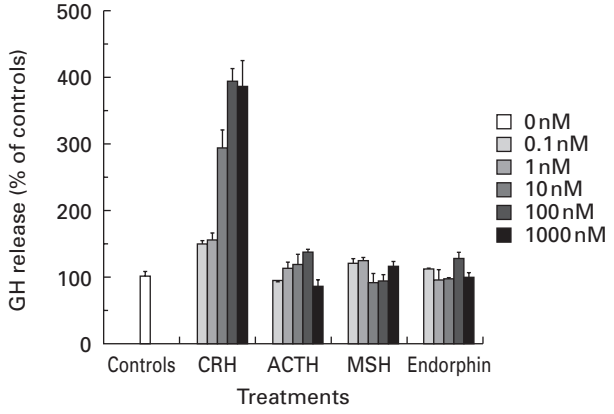

FIG. 5. Comparative effects of CRH and POMC-peptides (ACTH, α-MSH and β-endorphin) on GH release by eel pituitary cells. Cells were treated for 24 h. Results are expressed as percentage of GH released by control cells. Values are given as means ± SEM (n = 6 wells; 62 500 cells/well).

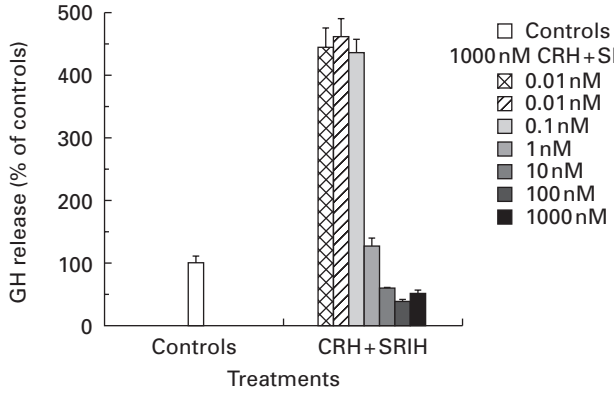

FIG. 6. Dose-dependent effect of SRIH on CRH-stimulated GH release by eel pituitary cells. Cells were treated for 24 h with 1000 nM CRH in presence or not of various doses (0.01–1000 nM) SRIH. Results are expressed as percentage of GH released by control cells. Values are given as means ± SEM (n = 6 wells; 62 500 cells/well).

the rainbow trout (40). Moreover, in the eel, CRH immunoreactivity in the brain and pituitary has been revealed using ovine CRH antiserum (41). In contrast, GHRH molecules isolated in vertebrates seem to share lower identities. For instance, catfish GHRH has only 58% identity to salmon and zebrafish GHRHs and 35–40% to human GHRH (13). Therefore, it cannot be excluded that the lack of effect of some neuropeptides such as GHRH could result from a strong species specificity of the molecule. However, heterologous GHRHs (carp or human GHRH) were reported to stimulate GH release in the goldfish, rainbow trout and tilapia (13), whereas homologous GHRH had no dose-dependent stimulatory effect in the salmon, *O. nerka* (18).

In other vertebrates, there are a few reports on the role of CRH in GH regulation. Secretion of GH by incubated pituitaries from hatchling turtles (*Pseudemys scripta*) was stimulated by ovine CRH; however, CRH, which was active at 250 nM but not at 25 nM, was less efficient than TRH and rat GHRH in the same conditions (42, 43). A dose-dependent effect of CRH on GH release was also seen on perifused pituitaries from adult turtles (*Chrysemys picta bellii* and *Trachemys* (*P.*) *scripta elegans*) (44). Again in these experiments, CRH, which significantly stimulated GH release at 10 nM, was less potent than rat GHRH which was active at 1 nM (44). These data show that CRH acts as a growth hormone-releasing factor in reptiles, but with a lower potency than in the eel. In the rat, a direct pituitary action of CRH on GH release by somatotrophs has not been demonstrated (45, 46). Further investigations in various classes of vertebrates would indicate to what extent the role of CRH on GH release has been conserved during vertebrate evolution.

As we used a mixed pituitary cell population in our experiments, we had to consider the possibility that the stimulatory effect of CRH on GH release could be mediated by activation of other pituitary cells. Indeed, CRH is a well-known stimulator of the secretion of ACTH, α-MSH and β-endorphin *in vivo* and *in vitro* in mammals (47) and in non-mammalian species (teleosts (39, 40, 48); amphibians (49, 50); birds (51)). Furthermore, a GH-releasing effect of α-MSH, involving a direct pituitary site of action, has been reported in human and rat (52); in the bullfrog (*Rana catesbeiana*), ACTH (1–17), but not α-MSH, stimulates GH release from dispersed pituitary cells (53). On the basis of these data, we investigated the possible action of POMC-derived peptides, ACTH (1–17), α-MSH and β-endorphin on GH release. None of these peptides were able to modify GH release in the eel, demonstrating that the stimulatory effect of CRH on somatotrophs was not mediated by one of these peptides released by corticotrophs and melanotrophs. However, it cannot be excluded that some other paracrine factors, possibly produced under the action of CRH, could be involved in the activation of somatotrophs. Indeed, numerous paracrine factors have been shown to be potentially involved in intercellular communication within the anterior pituitary in mammals (54).

To investigate whether CRH stimulatory action was mediated through binding to specific receptors, we used α-helical CRH (9–41), an analogue of CRH which antagonizes CRH-stimulated ACTH release in the rat (33) and in the goldfish (34). Our results showed that this antagonist inhibited the stimulatory effect of CRH on GH release by eel pituitary cells, causing a parallel rightward shift in the CRH dose–response curve similar to that observed for ACTH release in the rat (33). This indicates that, in the eel, CRH acts via receptors closely related to mammalian pituitary ones. Two subtypes of the CRH receptor have been identified in mammals: CRH1 (55) and CRH2, the latter being found in two different spliced forms, CRH 2α and CRH 2β (56). Recently, it has been shown that the structures of the two CRH receptors are highly conserved during evolution, since proteins found in the chum salmon (*O. keta*) exhibited 80% identity to the rat CRH1 receptor and 78% and 77% identity, respectively, to the rat CRH2α and CRH2β receptors (57). *In-situ* hybridization studies have shown the CRH1 receptor but not the CRH2 receptor subtypes in the rat pituitary (56). However, in fish, the type(s) of CRH receptor expressed in the pituitary have not yet been examined.

In the present study, the stimulatory action of CRH on GH release underwent desensitization. The effect of CRH on GH rate of release (ng/h) was reduced after 24 h and no significant effect, compared to controls, was observed at 96 h. In the rat, pretreatment of anterior pituitary cells with CRH reduced the ability of CRH to re-stimulate ACTH release. This desensitization involved an uncoupling of the CRH receptor from adenylate cyclase and/or a down-regulation of CRH receptors (58). A similar loss of responsiveness occurs for GH release after chronic exposure of cultured rat anterior pituitary cells to GHRH, and was associated with down-regulation of GHRH-binding sites (59). Similar mechanisms could be involved in the desensitization of GH response to CRH in the eel. In mammals, desensitization of pituitary hormone secretion in response to neurohormones has been related to a pulsatile pattern of stimulation (1). Recent studies have indicated a pulsatile secretion of GH in teleosts (carp, *Ctenopharyngodon idellus* (60); rainbow trout (61)).

Desensitization to PACAP also occurred after 24 h (data not shown). In contrast, there was no desensitization to the inhibitory effect of SRIH in the same experiments over 96 h. Furthermore, we have previously shown that SRIH was able to significantly inhibit GH release without desensitization for at least 12 days in culture, suggesting a potential chronic inhibitory regulation of GH by SRIH (12). The present study demonstrates that in addition, SRIH strongly inhibits the stimulatory effect of CRH on eel GH release. The overriding nature of SRIH control was also observed against the stimulatory action of PACAP (data not shown). Thus, we hypothesize that the neuroendocrine regulation of GH release in the eel involves a chronic predominant negative control by SRIH, and that pulsatile stimulatory controls by factors such as CRH and PACAP will only enter into play when SRIH inhibition is relieved.

Recent studies in teleosts indicated that CRH, in addition to its releasing action on POMC-peptides, could regulate the release of other pituitary hormones. In the rainbow trout, CRH stimulates *in vitro* the secretion of somatolactin (which belongs to the prolactin/GH family), but the effect was not dose-dependent (62). In the coho salmon (*O. kisutch*), CRH dose-dependently stimulated thyrotropin (TSH) secretion by pituitary cell cultures, and this effect was inhibited by the CRH antagonist α-helical CRH(9–41) (63). In the European

eel, CRH has also been found to increase TSHβ messenger RNA levels in our cell culture conditions (Pradet-Balade *et al*. unpublished data). In reptiles, CRH stimulates both GH and TSH releases (hatchling turtle (42, 43)).

Thus, CRH may be considered as a potential coordinator for activating different neuroendocrine axes. In lower vertebrates, CRH could be implicated in the regulation of critical developmental events, such as metamorphosis in amphibians and smoltification in salmonids, during which plasma levels of various hormones (namely cortisol, thyroid hormones, and GH) increase strongly (64–66). Silvering (second metamorphosis) in the eel is closely related to these developmental processes and could involve the co-ordinated actions of these hormones. In salmonids, GH and cortisol have been shown to act in synergy during osmoregulation: both GH and cortisol levels are elevated when hypoosmoregulatory ability is high (64), and GH and cortisol interact in stimulating gill Na^+, K^+-ATPase activity and salinity tolerance (67, 68). CRH could be implicated in this coordinated regulation of GH and cortisol for osmoregulation in teleosts. Moreover, in teleosts, activation of the hypothalamic-pituitary-interrenal axis which results in increased cortisol secretion (69), as well as increase in GH levels (70, 71), are major features affected by stress. Cortisol stimulates lipolysis and neoglucogenesis in teleosts as in other vertebrates (72), and GH interacts with cortisol to stimulate lipid mobilization by enhancement of lipolysis in teleosts (73) as in mammals (74). In teleosts, large increases in GH levels are also induced by fasting (rainbow trout (75); European eel (31)). It is plausible that CRH could be responsible for these stress-or fasting-induced GH increased release.

In humans, stressful conditions such as resistance physical exercise (76), fasting (77, 78), and hypoglycemia (79), also result in increased GH secretion with an activation of adrenal corticoids production (80). Hypersecretion of ACTH, cortisol and GH are also observed in patients with major depression (81). CRH could be involved in such neuroendocrine changes. Indeed, repeated, low doses of CRH modulate spontaneous GH release in healthy subjects (82). Moreover, depressed patients show an abnormal positive responsiveness of GH secretion to CRH administration, which is not correlated with CRH-induced ACTH and cortisol response (83). CRH has also been reported to increase GH secretion in patients with acromegaly (84). This suggests that CRH could act as a GH-releasing factor in human, at least in some physio-pathological conditions.

In contrast, in the rat, CRH seems to be rather implicated in an indirect inhibitory control of GH secretion. CRH inhibits GH release *in vivo* (85, 86) but not *in vitro* (45). The effect of CRH *in vivo* could result from stimulation of SRIH neurones (87) and inhibition of GHRH neurones (46). In opposition to their effects in teleosts and humans, stress and starvation result in a marked decrease in GH secretion in the rat (88–90). CRH can mediate the inhibitory action of stress on GH secretion, most possibly via an increased release of SRIH (91).

In conclusion, these data indicate that, whereas the inhibitory control of GH release by SRIH has been conserved throughout vertebrate evolution, the neuropeptides involved in the stimulatory control vary greatly according to species and/or physiological situations. Our results show a stimulatory effect of CRH on GH release in the eel, and this effect is played out directly at the pituitary cell level. This study in a primitive teleost suggests that CRH may have played an important early role as a co-ordinator in the activation of various endocrine axes involved in metamorphosis, osmoregulation, stress and fasting. The stimulatory role of CRH on GH release may have been partially conserved during evolution, as it is found in some human physio-pathological situations such as stress, fasting and depression.

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