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THE INHIBITORY EFFECTS OF γ-AMINOBUTYRIC ACID (GABA) ON GROWTH HORMONE SECRETION IN THE GOLDFISH ARE MODULATED BY SEX STEROIDS

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Summary

Double-labelling studies at the electron microscopic level demonstrated that γ-aminobutyric acid (GABA)-immunoreactive nerve endings are associated with growth-hormone-secreting cells in the proximal pars distalis of the goldfish pituitary gland, suggesting that GABA may be important for the control of growth hormone release in this species. An *in vitro* assay for GABA-transaminase activity demonstrated that the pituitary is a site for the metabolism of GABA to succinic acid. *In vitro*, GABA or the GABA antagonists bicuculline and saclofen did not affect the rate of growth hormone release from dispersed pituitary cells in static incubation. In contrast, intracerebroventricular injection of GABA reduced serum growth hormone levels within 30 min. During the seasonal gonadal cycle, intraperitoneal injection of GABA was without effect in sexually regressed goldfish, but caused a significant decrease in serum growth hormone levels in sexually recrudescent animals. Intraperitoneal implantation of solid silastic pellets containing oestradiol increased serum GH levels fivefold in sexually regressed and recrudescent goldfish; in both groups, GABA suppressed the oestradiol-stimulated increase in circulating growth hormone levels. The effect of oestradiol on basal serum growth hormone levels was specific since progesterone and testosterone were without effect. However, in recrudescent animals treated with progesterone and testosterone, the inhibitory effects of GABA on serum growth hormone levels were absent, indicating a differential role for these steroids in growth hormone release. Taken together, these results demonstrate that GABA has an inhibitory effect on growth hormone release in goldfish.

Key words: GABA, γ-aminobutyric acid, immunocytochemistry, growth hormone, oestradiol, goldfish, *Carassius auratus*.

Introduction

Growth is seasonally regulated in goldfish, and the highest growth rates are found in the early summer after the spring breeding period (Marchant and Peter, 1986). Growth hormone secretion from the goldfish anterior pituitary also varies seasonally (Marchant and Peter, 1986; Trudeau et al., 1992). Serum growth hormone levels increase during gonadal development in late autumn and winter, a time when somatic growth is lowest (Marchant and Peter, 1986; Trudeau et al., 1992). This increased release of growth hormone probably acts in concert with gonadotropin-II (GTH-II; the luteinizing-hormone-like molecule in fish) to stimulate steroidogenesis (Van Der Kraak et al., 1990; Le Gac et al., 1993) during seasonal redevelopment of the gonad.

The control of growth hormone release involves both stimulatory and inhibitory mechanisms. Growth hormone release in the goldfish is stimulated by bombesin (Himick and Peter, 1994), dopamine (Chang et al., 1985, 1990; Wong et al., 1993), thyrotropin-releasing hormone (TRH; Trudeau et al., 1992), neuropeptide Y (NPY; Peng et al., 1993), growth hormone-releasing hormone (GHRH; Vaughan et al., 1992), gonadotropin-releasing hormone (GnRH; Marchant et al., 1989a), cholecystokinin (Himick et al., 1993) and pituitary adenylate cyclase-activating polypeptide (PACAP; Wong et al., 1998). In contrast, growth hormone release is inhibited by insulin-like growth factor-1 (IGF-1; Weil et al., 1999), norepinephrine (Chang et al., 1985), serotonin (Somoza and Peter, 1991) and somatostatin (SRIF; Marchant et al., 1987). Interactions among these stimulatory and inhibitory neuroendocrine systems drive seasonal cyclicity in serum growth hormone levels (for reviews, see Peter and Marchant, 1995; Trudeau, 1997).

In contrast to the peptidergic and aminergic regulation of growth hormone release in fish, little information is available concerning the involvement of amino acid neurotransmitters. Glutamate, which is converted to γ-aminobutyric acid (GABA) by two molecular forms of glutamic acid decarboxylase in the

brain (Bosma et al., 1999; Pinal and Tobin, 1998), is localized in nerve terminals innervating the goldfish anterior pituitary. Activation of the *N*-methyl-D-glutamate (NMDA)-type glutamate receptor rapidly inhibits growth hormone release in this species (Trudeau et al., 1996). The effects of GABA on growth hormone release have not been studied in any species of fish; however, cell bodies containing GABA are located in hypophysiotrophic areas of the goldfish brain known to be involved in the control of growth hormone release (Martinoli et al., 1990). Moreover, GABA is an important neuroendocrine regulator of the release of other pituitary hormones in fish: GABA has prominent stimulatory effects on GTH-II release (Kah et al., 1992; Sloley et al., 1991; Trudeau et al., 1993a,b; Mañanos et al., 1999) and inhibitory effects on prolactin release (Prunet et al., 1993). In mammals and birds, GABA has been reported to have both stimulatory and inhibitory effects on growth hormone release. In the rat, for example, early reports indicated that central injection of GABA can either stimulate (McCann and Rettori, 1988) or inhibit (Fiók et al., 1984) growth hormone release in adults, depending on the site of application. In addition, GABA has a direct stimulatory effect on growth hormone release *in vitro* from pituitaries of young rats, whereas GABA has no effect or only slightly stimulates growth hormone release in adult rats (Fiók et al., 1984; Ács et al., 1990). The direct stimulatory effect of GABA in neonatal rats is independent of adrenal, gonadal and thyroid hormones (Ács et al., 1993). In the chicken, *in vitro* tissue culture experiments show that GABA inhibits growth hormone release in the presence of hypothalamic tissue but not directly at the pituitary (Hall et al., 1984). These interesting and conflicting reports led us to test whether GABA has any role in regulating growth hormone release in goldfish.

Materials and methods

Experimental animals

Common goldfish (*Carassius auratus*) weighing 15–40 g were purchased throughout the year from commercial suppliers. Fish were acclimated to 17 °C, and fed and maintained on a simulated natural photoperiod as reported previously (Trudeau et al., 1991). Fish were anaesthetized by immersion in 0.05 % tricaine methane sulphonate (TMS) prior to any handling for drug injection, steroid pellet implantation and blood sampling. Blood samples were taken by puncture of the caudal vasculature using a 25 gauge needle attached to a 1 ml syringe. Blood was allowed to clot for 16–24 h, and serum was collected by centrifugation.

Localization of GABA in the pituitary

Animals were anaesthetized with TMS and decapitated. The pituitaries were rapidly dissected, fixed with 6.5 % glutaraldehyde in phosphate buffer and cut at a thickness of 40 μm with a vibratome. The sections were processed for GABA pre-embedding immunohistochemistry as described previously (Kah et al., 1987a, 1992). For electron microscopy, the sections were flat-embedded, and fragments of interest were cut to obtain ultrathin sections. These sections were exposed to salmon growth hormone antibodies and, after rinsing, to a secondary antibody coupled to 20 nm gold particles (Kah et al., 1987a, 1992).

Metabolism of GABA in the pituitary

An *in vitro* enzyme assay was used to determine whether the pituitary was a site for GABA metabolism. Extraction procedures and determination of GABA-transaminase activity were performed as described by Sloley and McKenna (1993) except that a pooled homogenate of 10 pituitaries was incubated for 1 h before separation of ^{3}H-labelled metabolites using a tri-octylamine solution. Radioactivity was measured by pipetting 35 μl of supernatant into 4 ml of scintillation fluid (Packard Pico-Fluor 40) and counting in a Packard Tri-Carb liquid scintillation analyser. The specificity of GABA-transaminase activity was determined (in triplicate) by incubating pituitary extracts in the presence of increasing concentrations (0.01–100 μmol l^{-1}) of the GABA-transaminase inhibitor γ-vinyl-GABA (GVG; a gift from Hoechst Marion Roussel). GVG was added to pituitary extracts (on ice), incubated for 10 min at 37 °C and then for 5 min on ice before addition of [^{3}H]GABA (Amersham). The effectiveness of GVG in inhibiting GABA-transaminase activity was determined by calculating the dose giving 50 % inhibition (IC_{50}) using the Prism 2 program (GraphPad Software, Inc.).

The effects of GABA on growth hormone release from dispersed pituitary cells in vitro

To test whether the effects of GABA in inhibiting growth hormone release were direct at the level of the pituitary, static incubation of pituitary cells was carried out as described by Chang et al. (1990). For a given experiment, 40 pituitaries were collected from mixed populations of male and female goldfish, and their cells were dispersed by controlled trypsinization. Dispersed cells were resuspended in culture medium (Medium 199 with Earle's salts, Gibco, and containing 2.2 g l^{-1} $NaHCO_3$, 25 mmol l^{-1} Hepes and 1 % horse serum, pH 7.2) and cultured (2.5×10^{5} cells ml^{-1}) overnight in 24-well culture plates, with or without 100 000 units l^{-1} penicillin and 100 mg l^{-1} streptomycin. Cells were incubated at 28 °C, under 5 % CO_2 and at saturated humidity. On the following day, the culture medium was replaced with testing medium (Medium 199 with Hank's salts, Gibco, and containing 2.2 g l^{-1} $NaHCO_3$, 25 mmol l^{-1} Hepes and 0.1 % bovine serum albumin, pH 7.2), with or without 100 000 units l^{-1} penicillin and 100 mg l^{-1} streptomycin. A 0.1 mol l^{-1} GABA stock solution was made up in distilled deionized water and diluted in testing medium to give final concentrations of 0.001–100 μmol l^{-1} immediately prior to use. In another experiment, cells were incubated with 10 μmol l^{-1} GABA, in the presence or absence of 100 μmol l^{-1} of the $GABA_A$ antagonist bicuculline or 100 μmol l^{-1} of the $GABA_B$ antagonist saclofen. GABAergic drugs for cell culture and *in vivo* experiments were purchased from Research Biochemicals International (RBI). Following an additional 2 h of incubation under the conditions described

above, 750–800 µl of medium was carefully removed from each well and stored at −20 °C until the growth hormone contents were measured by radioimmunoassay. Treatments were usually tested in triplicate. Growth hormone levels from replicate experiments were normalized by expressing the data as a percentage of basal growth hormone release. The viability and responsiveness of cells were confirmed in parallel experiments with the same cell preparations using dopamine, the protein kinase C activator 4-β-tetradecanoyl phorbol acetate and cGnRH-II, agents known to stimulate growth hormone release (Chang et al., 1994).

The effects of injection of GABA into the third brain ventricle on growth hormone release in vivo

For injections into the third brain ventricle, 50 µg of GABA was delivered in 2 µl of saline to sexually regressed female goldfish. These doses of GABA were chosen because we have shown that they stimulate GTH-II release *in vivo* in goldfish (Trudeau et al., 1993b). To control for possible non-specific effects, the amino acid taurine (50 µg per 2 µl of saline) was also injected intracerebroventricularly. Taurine is abundant in the brain and can stimulate GTH-II release in goldfish (Sloley et al., 1991); it shares some of the characteristic inhibitory effects of GABA on neurotransmission (Huxtable, 1989). In addition, the relative molecular masses of taurine and GABA are similar and are, respectively, 125 and 103. Animals (25–35 g body mass) were anaesthetized with TMS and placed in a goldfish stereotaxic apparatus (Peter and Gill, 1975); the skull was then opened using a circular dental saw. The injection syringe was gently lowered into the third brain ventricle, and 2 µl of injection fluid was expelled by light pressure. The syringe was removed, and the fish was returned to the experimental tank for recovery (<5 min) from the anaesthetic. Blood samples were drawn 30 min after intracerebroventricular injection.

The effects of GABA and sex steroids on growth hormone release

Preliminary studies indicated that the inhibitory effects of GABA on growth hormone were dependent on gonadal status, implicating sex steroids in the control of growth hormone release. Gonad-intact, sexually regressed or sexually recrudescent goldfish were implanted for 5 or 10 days with control or silastic pellets containing progesterone, testosterone or oestradiol (100 µg g^{-1} body mass), as reported by Trudeau et al. (1991). On the day of experimentation, GABA dissolved in saline (100 µg g^{-1} body mass; purchased from RBI) or saline control (5 µl g^{-1} body mass) was injected intraperitoneally, and blood samples were taken 30 min later. This dose of GABA and sampling times were based on a preliminary study (not shown) and also on our previous work in goldfish in which similar doses of GABA stimulated GTH-II release within 30 min (Trudeau et al., 1993b).

Radioimmunoassay

Serum or culture medium growth hormone levels were measured using a double-antibody radioimmunoassay (RIA) (Murthy et al., 1993) with common carp growth hormone as standard. All samples were assayed in duplicate.

Statistical analyses

Growth hormone concentrations in serum or *in vitro* incubation medium were analysed by one-way analysis of variance or Student's *t*-test; treatment group means were considered statistically different at $P<0.05$.

Results

Immunohistochemical localization of GABA in the pituitary

The anterior pituitary of teleosts has the unique property of receiving direct innervation, which is the functional equivalent of the median eminence of tetrapods (Peter et al., 1990). Furthermore, endocrine cells of the same type are more or less grouped in specific portions of the anterior pituitary, allowing the nature of the innervation of a given cell type to be easily determined. At the light microscope level (not shown), GABA-immunoreactive fibres were observed entering the pituitary stalk and digitating in all lobes of the pituitary, notably the proximal pars distalis, which contains mainly gonadotrophs and somatotrophs, confirming our previous studies (Kah et al., 1987a, 1992). At the electron microscope level (Fig. 1), these fibres were detected in the neurohypophyseal digitations, but also in direct contact with the secretory cells. Double-staining studies at the electron microscope level demonstrated that GABA-immunoreactive nerve profiles were frequently associated with growth-hormone-positive cells. These nerve profiles contained positive neurosecretory granules 60–80 nm in diameter and, although no clear synaptic differentiation could be detected apposed to the growth hormone cells, membrane thickenings facing secretory cells and synaptic-like vesicles were occasionally detected in the positive nerve profiles.

Metabolism of GABA in the pituitary

The presence of GABAergic nerve terminals in the pars distalis suggested that GABA may be released and metabolized in the pituitary. A sensitive *in vitro* assay demonstrated GABA-transaminase activity in the goldfish pituitary. γ-Vinyl-GABA (GVG), previously characterised as a GABA metabolism inhibitor *in vivo* in the goldfish (Sloley et al., 1991), effectively inhibited specific GABA-transaminase activity *in vitro* (Fig. 2). The IC_{50} for GVG inhibition of goldfish pituitary GABA-transaminase was 1.4 µmol l^{-1}; 100 % inhibition was obtained using the highest dose (100 µmol l^{-1}) tested.

The effects of GABA on growth hormone release from dispersed pituitary cells in vitro

Detection of a GABAergic innervation and specific GABA-transaminase activity in the pituitary suggested that

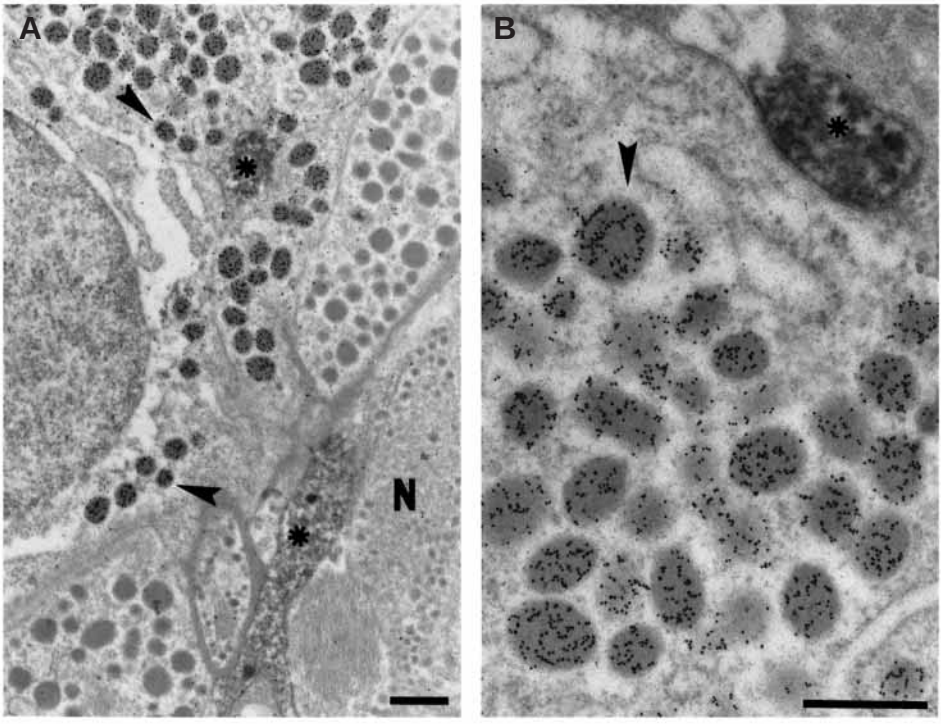

Fig. 1. (A) Electron micrograph at the level of the proximal pars distalis of the pituitary showing the presence of γ-aminobutyric acid (GABA)-positive profiles (*) located either in digitations of the neurohypophysis (N) or in direct contact with growth-hormone-positive cells (arrowheads). Scale bar, 0.5 µm. (B) High-power view of a GABA-positive nerve ending (*) in close association with a growth-hormone-positive cell. Note the strong labelling of the secretory vesicles (arrowhead) by gold particles. Scale bar, 0.5 µm.

GABA may directly regulate growth hormone release in goldfish. *In vitro* and *in vivo* approaches were used to address this possibility. Incubation of dispersed pituitary cells with 1–100 µmol l^{-1} GABA had no effect on *in vitro* growth hormone release (Fig. 3A). The viability and responsiveness of the cultured growth hormone cells were confirmed by high levels of basal growth hormone release, and growth-hormone-release responses to dopamine and 4-β-tetradecanoyl phorbol acetate (data not shown), consistent with our extensive data on growth hormone release *in vitro* (Chang et al., 1994). In this experiment, the presence of penicillin and streptomycin in the culture medium was potentially a concern since penicillin may act as a GABA antagonist in some circumstances (Macdonald and Olsen, 1994). However, a second experiment using dispersed cells cultured in the complete absence of penicillin and streptomycin also indicated that GABA does not directly affect growth hormone release (Fig. 3B). In this experiment, high basal levels of release under control conditions were also noted. Incubation of cells with 1 nmol l^{-1} to 10 µmol l^{-1} GABA did not affect growth hormone release (P>0.05). In another experiment (Fig. 3C), the $GABA_A$ and $GABA_B$ receptor antagonists bicuculline and saclofen, respectively, did not affect growth hormone release either alone or in combination with GABA (P>0.05). Cell viability and secretory responses were confirmed by growth-hormone-release responses to 100 nmol l^{-1} chicken GnRH-II (control 100±3.5 %; chicken GnRH-II, 113.2±3.9 %, P<0.05; not shown).

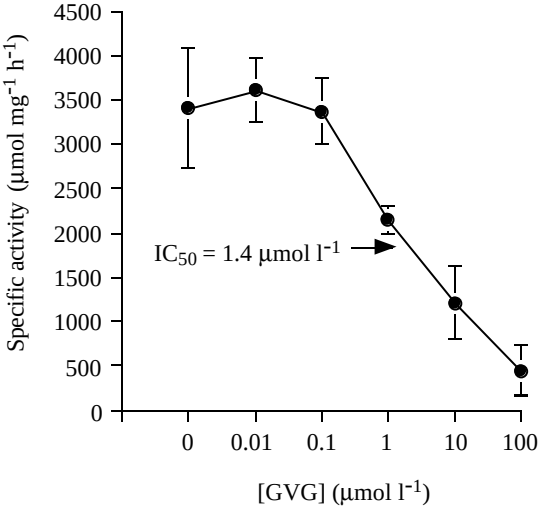

Fig. 2. Specific γ-aminobutyric acid (GABA)-transaminase activity in the goldfish pituitary. Increasing concentrations of γ-vinyl-GABA (GVG) inhibit GABA-transaminase activity. Values are presented as means ± S.E.M. (N=3).

The effects of injection of GABA into the third brain ventricle on growth hormone release in vivo

Fig. 4 shows the effect of injection of GABA into the third brain ventricle on serum growth hormone levels in fish in post-spawning condition (at the beginning of gonadal regression). At 30 min following central injection, GABA (50 µg) suppressed serum growth hormone levels by approximately 23 % (Fig. 4). In contrast, taurine did not affect growth hormone levels.

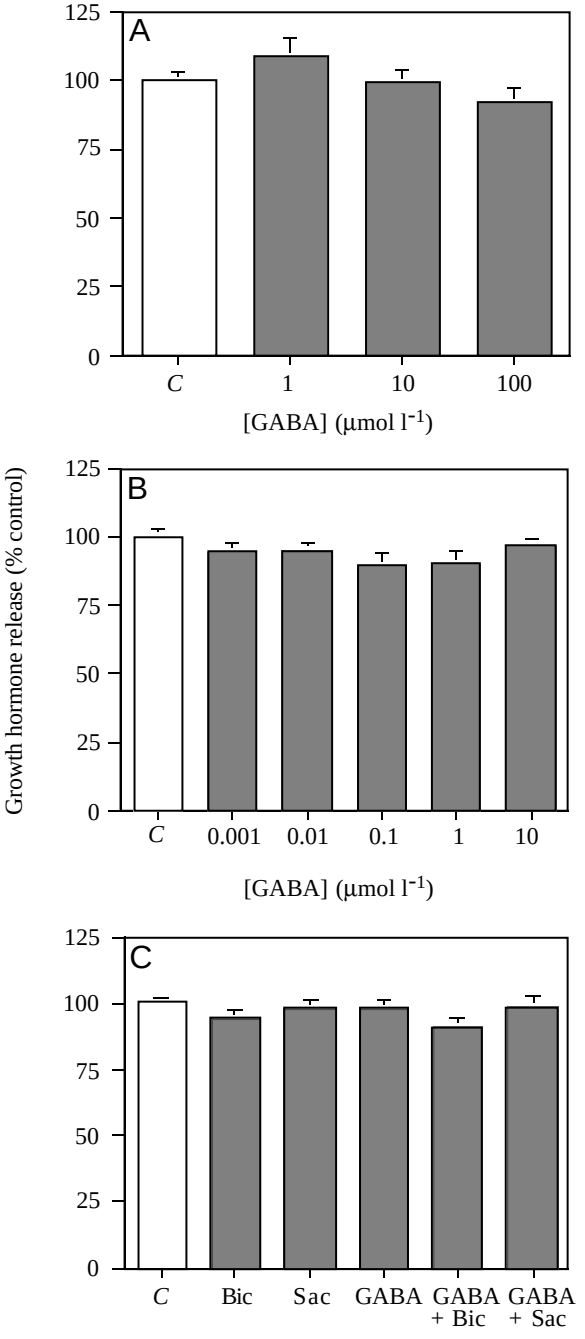

Fig. 3. The effect of γ-aminobutyric acid (GABA) on the *in vitro* release of growth hormone (GH) from dispersed goldfish pituitary cells. (A) Values are means + S.E.M. (N=8–9) and are expressed as a percentage of control (C) basal GH levels (1179±61 ng ml^{-1}). Cells derived from pituitaries of sexually mature fish were incubated in the presence of penicillin and streptomycin. (B) Values are + S.E.M. (N=12) and are expressed as a percentage of control (C) basal GH levels (1062±33 ng ml^{-1}). Cells derived from pituitaries of sexually regressed fish were incubated in the absence of penicillin and streptomycin. (C) Values are + S.E.M. (N=12) and are expressed as a percentage of control (C) basal GH levels (1143±24 ng ml^{-1}). Cells derived from pituitaries of sexually regressed fish were incubated in the absence of penicillin and streptomycin. Drug concentrations used were 10 μmol l^{-1} GABA, 100 μmol l^{-1} Bicuculline (Bic) and 100 μmol l^{-1} Saclofen (Sac).

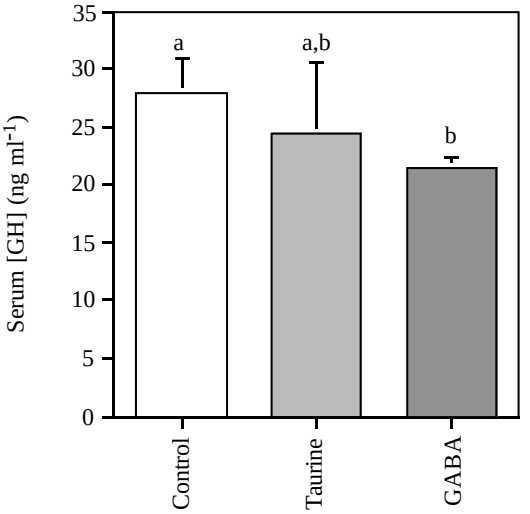

Fig. 4. The effects of injection of γ-aminobutyric acid (GABA) (50 μg in 2 μl of saline) and taurine (50 μg in 2 μl of saline) into the third brain ventricle on serum growth hormone (GH) levels in sexually regressed female goldfish. Values are + S.E.M. (N=11). Means with different superscripts are significantly different (P<0.05).

The effects of GABA and sex steroids on growth hormone release

In sexually regressed goldfish implanted with control silastic pellets, GABA injected intraperitoneally (100 μg g^{-1} body mass) did not affect serum growth hormone levels (Fig. 5). We reasoned that this lack of effect of GABA might be indicative of the decreased growth hormone secretory response associated with sexual regression and low sex steroid levels in the summer (Trudeau et al., 1992). Therefore, sexually regressed goldfish were treated for 5 days with silastic implants containing testosterone or oestradiol. Testosterone alone had no effects on serum growth hormone levels, and GABA had no effect in the testosterone-treated group. In contrast, oestradiol treatment caused an approximately fivefold increase in serum growth hormone levels (Fig. 5). In the oestradiol-implanted group, intraperitoneal injection of GABA suppressed growth hormone release by 50 %.

The effects of intraperitoneal injection of GABA in fish in the early stages of seasonal gonadal redevelopment (recrudescence) were also tested. In these animals, GABA suppressed growth hormone release by 50 % within 30 min of injection (Fig. 6). Treatment with progesterone or testosterone alone for 10 days had no effects on basal serum growth hormone levels compared with fish implanted with silastic pellets without steroid. However, the inhibitory effects of injected GABA on serum growth hormone levels found in control implanted (no steroid) recrudescent fish were absent in the progesterone- and testosterone-treated fish. Oestradiol treatment again increased serum growth hormone levels fivefold, and GABA injection suppressed this stimulated release by approximately 70 % (Fig. 6).

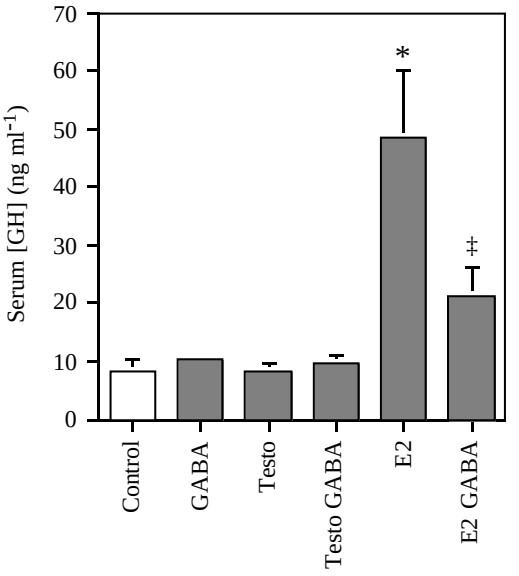

Fig. 5. The effects of γ-aminobutyric acid (GABA) (100 μg g^{-1} body mass) on growth hormone (GH) release in sexually regressed female goldfish implanted for 5 days with testosterone (Testo) or oestradiol (E2). *P<0.01, oestradiol stimulates GH release. ‡P<0.05, GABA inhibits GH release in oestradiol-treated fish. Values are means + S.E.M. (N=10–12). Note that the error for the group treated with GABA alone is too small to observe.

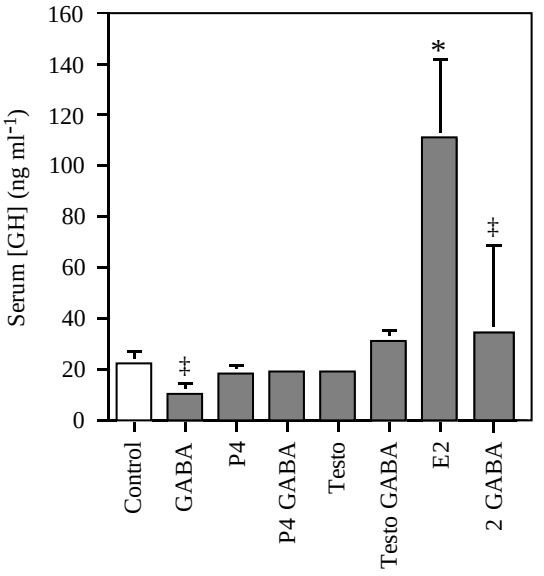

Fig. 6. The effect of γ-aminobutyric acid (GABA) (100 μg g^{-1} body mass) on growth hormone (GH) release in sexually recrudescent female goldfish implanted for 10 days with testosterone (Testo), progesterone (P4) or oestradiol (E2). *P<0.01, oestradiol stimulates GH release. ‡P<0.05, GABA inhibits GH release in control and oestradiol-treated fish. Values are means + S.E.M. (N=10–12). Note that the errors for the groups treated with P4 plus GABA or testosterone alone are too small to observe.

Discussion

The presence of GABA and the GABA-metabolising enzyme GABA-T in the pituitary

The goldfish hypothalamus and pituitary contain high levels of GABA as determined by HPLC analysis (Sloley et al., 1991). Double-labelling electron microscopic studies demonstrated that GABA-producing neurons innervate that part of the anterior pituitary where somatotroph cells are located (Fig. 1). The origin of this GABAergic innervation is unknown. However, retrograde tracing studies have shown that the preoptic region and the mediobasal hypothalamus, both of which exhibit high densities of GABA-immunoreactive cell bodies (Martinoli et al., 1990), are the main hypophysiotrophic regions in the goldfish brain (Anglade et al., 1993). We have previously demonstrated that these GABA neurons are important for the neuroendocrine control of pituitary function because injection of the GABA-transaminase inhibitor GVG raises GABA levels by approximately threefold in the preoptic region, hypothalamus and pituitary, leading to the upregulation of pituitary GTH-II β-subunit mRNA levels (Trudeau et al., 2000) and release of GTH-II *in vivo* (Trudeau et al., 1993b). We also show that the pituitary contains GABA-transaminase (Fig. 2) activity, indicating that the pituitary is also an active site for the metabolism of GABA. The cellular localization of GABA-T in the pituitary remains to be determined.

GABA inhibits growth hormone release in vivo *in goldfish treated with oestradiol*

A few studies in rats have demonstrated that GABA can act directly at the anterior pituitary (Fiók et al., 1984; Ács et al., 1990, 1993), but also centrally (McCann and Rettori, 1988; Ács et al., 1993), to regulate growth hormone release. Despite the detection of GABA-immunoreactive nerve terminals apposed to growth-hormone-secreting cells, we were not able to show that GABA directly affected *in vitro* release of growth hormone from dispersed pituitary cells obtained from mature or regressed goldfish (Fig. 3). However, injection of GABA into the brain ventricle inhibited release of growth hormone in post-spawning goldfish (Fig. 4), implicating GABA in the control of growth hormone secretion in this species. An inhibitory effect was not seen when GABA was injected intraperitoneally in sexually regressed fish (Fig. 5). This effect could have been dose-related, although 100 μg g^{-1} GABA is effective in stimulating GTH-II release in sexually regressed goldfish (Trudeau et al., 1993b). In contrast, the 100 μg g^{-1} dose of GABA inhibited growth hormone release when injected intraperitoneally into fish in the early stages of gonadal recrudescence (Fig. 6), suggesting that gonadal steroids may modulate the action of GABA.

Indeed, GABA clearly inhibited growth hormone release in both regressed and recrudescent animals treated with oestradiol to increase basal levels of growth hormone secretion (Figs 5, 6). This action of GABA in inhibiting growth hormone release within 30 min is very robust considering that oestradiol increases pituitary growth hormone content and enhances

growth hormone secretion for at least 10 days (Zou et al., 1997). The effects of oestradiol on serum growth hormone levels were different from those of the other steroids tested. Progesterone and testosterone did not affect basal serum growth hormone levels. However, recrudescent animals treated with progesterone or testosterone did not respond to the inhibitory effects of GABA on serum growth hormone levels (Fig. 6), indicating a differential role for these steroids on growth hormone release. Contrasting effects of the sex steroids on GABA synthesis have also been observed in the goldfish hypophysiotropic system. For example, oestradiol increased, whereas both testosterone and progesterone decreased, GABA synthesis in the pituitary (Trudeau et al., 1993a).

The site of GABA action for inhibiting growth hormone release in vivo

GABA could be inhibiting growth hormone release either by direct actions on the somatotroph or indirectly *via* the release of other neurotransmitters or neuropeptides. GABA or GABA antagonists did not affect *in vitro* growth hormone release from dispersed pituitary cells that were responsive to GnRH. Lesioning of the preoptic region and basal hypothalamus has shown that, *in vivo*, growth hormone release in the goldfish is under tonic inhibition by somatostatin (Marchant et al., 1989b). In contrast, for pituitary cells *in vitro*, these predominant inhibitory influences have been removed. Therefore, dispersed pituitary cells of the goldfish typically secrete high levels of growth hormone *in vitro*. In this situation of high basal release, GABA did not inhibit growth hormone release over a wide range of doses administered *in vitro* to goldfish pituitary cells in static culture. In contrast, intracerebroventricular injection of GABA reduced serum growth hormone levels. In Atlantic salmon, at least, $GABA_A$ receptor subunits have been demonstrated immunohistochemically throughout the preoptic–hypophysiotropic system (Anzelius et al., 1995). Using patch-clamp electrophysiology, we have demonstrated functional inhibitory $GABA_A$ receptors in the ventral preoptic area of goldfish (Trudeau et al., 2000). In goldfish, GABA injected into the third brain ventricle could activate preoptic GABA receptors to decrease serum growth hormone levels, suggesting a central site of action. However, the modest reduction of growth hormone levels following intracerebroventricular injection compared with the more obvious effects of intraperitoneally injected GABA suggest that GABA may be acting predominantly on hypophysiotrophic nerve terminals rather than centrally. Alternatively, intracerebroventricularly injected GABA could be rapidly degraded by GABA-T or removed from interstitial spaces in the brain by GABA transporters, thus attenuating the growth-hormone-release response. Although we cannot yet entirely rule out a direct action of GABA on somatotrophs under some other physiological conditions that we have not yet tested, the currently available data suggest that GABA probably acts indirectly, through unidentified neuronal systems, to inhibit growth hormone release. Studies in the chicken also demonstrate that the inhibitory effects of GABA on growth hormone release are indirect (Hall et al., 1984).

Possible mechanisms for GABAergic inhibition of growth hormone release

Studies in the rat indicate that GABA can stimulate growth hormone release by inhibiting somatostatin-secreting neurons (McCann and Rettori, 1988). In goldfish, a similar effect of GABA on somatostatin release is not a likely mechanism for the GABAergic inhibition of growth hormone release observed here. An inhibition of somatostatin release would lead to stimulation rather than inhibition of growth hormone release in the goldfish model. In goldfish, GABA has been shown to stimulate GnRH release (Kah et al., 1992), and GnRH stimulates growth hormone release (Marchant et al., 1989a), especially in oestradiol-treated animals (Trudeau et al., 1992). These observations do not support the involvement of GnRH in the GABAergic inhibition of growth hormone release.

GABA could also act by suppressing dopaminergic activity. We have previously shown that GABA can inhibit dopamine turnover in the goldfish hypothalamo–pituitary axis (Trudeau et al., 1993a), and dopamine, through the activation of a pituitary D1 receptor, stimulates growth hormone release (Wong et al., 1993; Chang et al., 1994). Preoptic dopamine neurones also innervate the anterior pituitary, in which the somatotrophs are localized (Kah et al., 1987b), and GABA/dopamine axo-axonal interactions are therefore possible. In a preliminary study (V. L. Trudeau, unpublished data), the GABA metabolism inhibitor GVG ($300\,\mu g\,g^{-1}$ body mass injected intraperitoneally) given alone had no effect on serum growth hormone levels compared with saline-injection in sexually regressed control fish. This is consistent with the lack of effect of intraperitoneally injected GABA in regressed fish documented in the present study. However, in the preliminary study, when GVG was given in combination with the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine ($240\,\mu g\,g^{-1}$ body mass injected intraperitoneally) to inhibit dopamine synthesis, a significant inhibitory effect on serum growth hormone levels was observed. It may be that the potent stimulatory dopamine input to growth-hormone-secreting cells overrides the inhibitory effects of GABA, especially in sexually regressed animals. Further analysis of GABA/dopamine interactions within the goldfish hypophysiotrophic system is clearly warranted.

The GABA receptor subtypes mediating inhibition of growth hormone release were not studied. Our previous work in goldfish indicated that GABA stimulated GTH-II release by activating the $GABA_A$-type receptor, and an additional stimulatory component dependent on the $GABA_B$ receptor was also evident (Trudeau et al., 1993b). These functional studies indicate the presence of the two GABA receptor subtypes, and their respective roles in controlling growth hormone release in the goldfish remain to be determined.

Concluding remarks

The present series of experiments suggests that GABA is

involved in the inhibitory control of growth hormone release. This contrasts with the stimulatory effects of GABA on GTH-II release in goldfish (Kah et al., 1992; Sloley et al., 1991; Trudeau et al., 1993a,b) and trout (Mañanos et al., 1999). GABAergic neurons projecting to somatotrophs and gonadotrophs (Kah et al., 1987a, 1992), therefore, differentially regulate the secretory activity of these two adjacent cell types within the anterior pituitary. Furthermore, we have previously demonstrated that GVG concomitantly upregulates the expression of mRNA for the secretory vesicle protein secretogranin-II (SgII) and decreases the cell content of GTH-II in gonadotrophs, indicating a GABAergic activation of a regulated secretory pathway (Blázquez et al., 1998). In contrast, in the same experiment, GVG did not alter somatotroph SgII mRNA levels, yet increased cell growth hormone content. We interpret this as inhibition of growth hormone secretion by GABA, which supports our results showing that GABA injections reduce serum growth hormone levels in maturing goldfish. We have also presented evidence that oestradiol modulates the GABAergic control of growth hormone release in goldfish. This latter observation is especially significant because it is known that growth hormone stimulates ovarian oestradiol production (Van Der Kraak et al., 1990; Le Gac et al., 1993) and oestradiol, in turn, enhances growth hormone production and release (Trudeau et al., 1992; Zou et al., 1997). Oestradiol can increase dopamine turnover in the goldfish hypothalamus and pituitary (Trudeau et al., 1993a), which would contribute to a positive feedback regulation of growth hormone release. Conversely, oestradiol increases hypothalamic and pituitary GABA synthesis (Trudeau et al., 1993a), and GABA inhibits growth hormone release in maturing or oestradiol-treated animals. We hypothesise that the GABAergic neurons in the preoptic region and/or hypothalamus are part of a gonadal feedback system controlling growth hormone secretion. Additional studies are required to characterize fully the receptor subtypes, site and mechanism of GABA action involved in the inhibition of growth hormone release.

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