Journal of Neurochemistry
Raven Press, Ltd., New York

Amino Acid Neurotransmitters and Dopamine in Brain and Pituitary of the Goldfish: Involvement in the Regulation of Gonadotropin Secretion

B. Duff Sloley, *Olivier Kah, Vance L. Trudeau, Joseph G. Dulka, and Richard E. Peter

*Department of Zoology, University of Alberta, Edmonton, Alberta, Canada; and *Laboratoire de Neurocytochimie Functionelle, URA CNRS 339, Université de Bordeaux I, Talence, France*

Abstract: An isocratic high-performance liquid chromatographic technique was developed to measure levels of γ-aminobutyric acid (GABA), glutamate, and taurine in the brain and pituitary of goldfish. Accuracy of this procedure for quantification of these compounds was established by evaluating anesthetic and postmortem effects and by selectively manipulating GABA concentrations by intraperitoneal administration of the glutamic acid decarboxylase (GAD) inhibitor 3-mercaptopropionic acid or the GABA transaminase inhibitor γ-vinyl GABA. The technique provided a simple, rapid, and reliable method for evaluating the concentrations of these amino acids without the use of complex gradient chromatographic systems. To investigate the relationship between neurotransmitter amino acids and the control of pituitary secretion of gonadotropin, the effects of injection of taurine, GABA, or monosodium glutamate on GABA, glutamate, taurine, and, in some instances, monoamine concentrations in the brain and pituitary were evaluated and related to serum gonadotropin levels. Injection of taurine caused an elevation in serum gonadotropin concentrations. In addition, injection of the taurine precursor hypotaurine but not the taurine catabolite isetheonic acid elevated serum gonadotropin levels. Intracerebroventricular injection of either GABA or taurine also elevated serum gonadotropin concentrations. Pretreatment of recrudescent fish with α-methyl-*p*-tyrosine reduced pituitary dopamine concentrations and also potentiated the serum gonadotropin response to taurine. Injection of monosodium glutamate caused an increase of glutamate content in the pituitary at 24 h; this was followed by a decrease at 72 h after administration. Pituitary GABA, taurine, and dopamine concentrations underwent a transient depletion after monosodium glutamate administration, and this was associated with an elevation of serum gonadotropin content. The increase in serum gonadotropin concentrations in response to a gonadotropin-releasing hormone analogue was potentiated by pretreatment with monosodium glutamate. This article demonstrates that procedures causing elevation in GABA and taurine concentrations stimulate gonadotropin release in a teleost fish. **Key Words:** γ-Aminobutyric acid—Glutamate—Taurine—Dopamine—Gonadotropin—Goldfish. **Sloley B. D. et al.** Amino acid neurotransmitters and dopamine in brain and pituitary of the goldfish: Involvement in the regulation of gonadotropin secretion. *J. Neurochem.* **58,** 2254–2262 (1992).

In teleost fish a considerable amount of evidence indicates that both neuropeptide and aminergic neurotransmitters are involved in the neuroendocrine control of gonadotropin (GTH) release. Regulation of GTH release involves both stimulatory and inhibitory factors. In the goldfish, dopamine (DA) directly inhibits GTH secretion by activation of DA type 2-like receptors (Peter et al., 1986; Chang et al., 1990). DA also inhibits GTH-releasing hormone (GnRH) release at both the pituitary and preoptic–anterior hypothalamic levels (Yu and Peter, 1990; Yu et al., 1991). GTH release is directly stimulated by at least two forms of GnRH (Peter et al., 1990). Norepinephrine also directly stimulates GTH release (Chang et al., 1991) and stimulates GnRH release from preoptic–anterior hypothalamic slices and pituitary fragments (Yu and Peter, 1990; Yu et al., 1991). Serotonin [5-hydroxytryptamine (5-HT)] stimulates GTH release

Received October 2, 1991; revised manuscript received November 8, 1991; accepted November 22, 1991.

Address correspondence and reprint requests to Dr. B. D. Sloley at Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada.

Abbreviations used: DA, dopamine; GABA, γ-aminobutyric acid; GnRH, gonadotropin-releasing hormone; GSI, gonadosomatic index; GTH, gonadotropin; 5-HT, 5-hydroxytryptamine (serotonin); 3-MPA, 3-mercaptopropionic acid; α-MPT, α-methyl-*p*-tyrosine; MSG, monosodium glutamate; sGnRHa, salmon gonadotropin-releasing hormone analogue.

in goldfish both in vivo (Somoza et al., 1988) and in vitro from perfused fragments of the pituitary (Somoza and Peter, 1991), but it is not known whether this is a direct stimulation of the gonadotrophs; 5-HT stimulates GnRH release from preoptic–anterior hypothalamic brain slices in static culture (Yu et al., 1991), indicating that GnRH release may be at least part of the stimulatory actions of 5-HT on GTH release in goldfish. Other factors, including opiates (Rosenblum and Peter, 1989) and neuropeptide Y (Peng et al., 1990), also stimulate GTH release in teleosts.

γ-Aminobutyric acid (GABA), glutamate, and taurine are considered important amino acid neurotransmitters in vertebrates, and GABA is implicated in the control of pituitary function (McCann et al., 1984; McCann and Rettori, 1988). To date there is very little information concerning amino acid neurotransmitters within the CNS and pituitary of teleost fish. In particular, GABA and taurine, which are found in high concentrations in mammalian brain and pituitary (Guidotti, 1978; Tappaz et al., 1983), have rarely been quantified in fish (Nilsson, 1990). Immunohistochemical evidence exists for GABAergic innervation of the teleost brain (Martinoli et al., 1990) and pituitary (Kah et al., 1987), but no information is available on the anatomical distribution of taurine in fish brain or pituitary. In mammals, both GABA (Anden and Stock, 1973; Biswas and Carlson, 1977) and taurine (Ahtee and Vahala, 1985; Kontro, 1987) have been demonstrated to alter DA function in brain. Considering both the importance of the inhibitory influences of DA on GnRH and GTH secretion in the goldfish and the potential for modulation of DA by GABA and taurine, investigation of the influences of these amino acid neurotransmitters on GTH secretion in fish is warranted.

This study presents data concerning the quantitative measurement of levels of amino acid neurotransmitters in various regions of the goldfish brain and pituitary. Several drugs known or believed to affect selectively GABA metabolism were also tested for effects on neurotransmitter amino acid concentrations and, in some cases, monoamine concentrations and serum GTH concentrations. This study provides an initial basis for evaluation of the role of amino acid neurotransmitters in the regulation of pituitary function in fish.

MATERIALS AND METHODS

Animals

Goldfish (*Carassius auratus;* common variety), 9–11 cm long, were purchased throughout the year from Ozark Fisheries (Stoutland, MO, U.S.A.). From the time of arrival, the fish were held on a simulated natural photoperiod (Edmonton, Alberta, Canada) in flow-through aquaria at 18 ± 1°C. Fish were fed once daily with commercial trout food.

Pharmacological agents and chemicals

DA HCl, norepinephrine HCl, 5-HT creatinine sulfate, GABA, taurine, hypotaurine, L-cysteic acid, isetheonic acid, β-alanine, L-lysine, D,L-homoserine, L-glutamic acid, α-methyl-*p*-tyrosine (α-MPT), monosodium glutamate (MSG), and 3-mercaptopropionic acid (3-MPA) were purchased from Sigma (St. Louis, MO, U.S.A.). Fluoraldehyde (*o*-phthalaldehyde reagent solution) was obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.). D,L-γ-Vinyl GABA (D,L-4-amino-hex-5-enoic acid) was the generous gift of the Merrell Dow Research Institute (Strasbourg, France). The salmon GnRH analogue (sGnRHa) [D-Arg^6,Pro^9-*N*-Et]-salmon GnRH was a gift from Dr. J. Rivier of The Salk Institute (La Jolla, CA, U.S.A.). Drugs were prepared fresh before injection in a vehicle of 0.6% NaCl except taurine, which was dissolved in distilled water. Control groups were given an equivalent volume of the appropriate vehicle. All chemicals used for analyses were reagent grade or better, and all solvents were HPLC grade. Water was distilled and deionized with a resistance of >17 MΩ.

Experimental procedures

The drugs were given as intraperitoneal or intracerebroventricular injections. Intracerobroventricular injections were included in the study to act as controls for nonspecific effects that might arise from the intraperitoneal injection of large amounts of amino acids. In addition, intracerebroventricular injection avoids loss of injected substance through the gills before the drug reaches the brain and circumvents the blood–brain barrier. Doses of drugs injected intraperitoneally are expressed as micrograms per gram of body weight and were administered in a 5-μl volume per gram of body weight, except taurine (10-μl volume per gram of body weight). Intracerebroventricular injections were performed by the method of Chang and Peter (1983). Control fish for the experiment investigating the effects of anesthesia were killed by severing the spinal cord just posterior to the head. All other fish were anesthetized by immersion in 0.05% tricaine methanesulfonate for between 1 and 2 min and weighed before dissection. Blood was collected by puncturing the caudal vasculature with a 25-gauge needle attached to a 1-ml disposable syringe. Blood samples were allowed to clot on ice for several hours, and the serum was separated by centrifugation and stored at −28°C. Serum GTH was quantified by radioimmunoassay (Peter et al., 1984). All samples were assayed in duplicate, and samples from the same experiment were measured in one assay.

Brains of fish were rapidly removed through an opening made in the top of the skull and placed on an ice-cold glass plate. The hypothalamus and telencephalon (including preoptic region) (Yu et al., 1987) were rapidly removed, placed in preweighed 1.5-ml polypropylene microtubes, and immediately frozen on dry ice. The pituitary was removed, placed in a 1.5-ml polypropylene microtube, and frozen on dry ice. Dissections took <2 min. Tissues were stored frozen at −28°C until estimation of amino acid or amine concentrations (usually <1 week). After removal of the brain, gonads were removed for calculation of the gonadosomatic index (GSI = gonad weight/body weight × 100).

Concentrations of GABA, glutamate, and taurine were determined by HPLC with fluorometric detection. Amino acids were extracted from tissues by homogenizing tissues, using an ultrasonic tissue disruptor, in 250 (hypothalamus and telencephalon) or 40 μl (pituitary) of methanol containing 0.1 μg/ml of homoserine as the internal standard. Homogenates were centrifuged for 10 min at 12,800 *g*, and 20 μl of the supernatant was removed and reacted with 20 μl of

fluoraldehyde solution (Pierce) for 2.5 min to produce fluorescent derivatives. Twenty microliters of the reaction product was mixed with 980 μl of 0.1 *M* sodium acetate, and 20 μl of this solution was injected directly onto the HPLC column. Separation of amino acid derivatives was achieved using isocratic reverse-phase liquid chromatography. The mobile phase consisted of 0.1 *M* NaH_2PO_4/methanol/acetonitrile (20:11:3 by volume). The pH was adjusted to 3.8 with concentrated phosphoric acid, degassed, and pumped at a flow rate of 1 ml/min. This mobile phase could be recycled for periods of up to 1 month with constant use. The apparatus for HPLC with fluorometric detection consisted of a Gilson model 305 pump equipped with a model 805 manometric unit and a Rheodyne manual injector equipped with a 20-μl injection loop. Separations were achieved using a 25-cm × 4.6-mm (inside diameter) analytical column packed with C-18 spherical 5-μm particles (Ultrasphere; Beckman) and protected by a 3-cm × 4.6-mm (inside diameter) guard column (MPLCRP-18 SPHERI-5; Brownlee). Fluoraldehyde derivatives of GABA, homoserine, glutamate, and taurine were detected by fluorometric detection using a Gilson model 121 fluorometer with an excitation wavelength of 305–395 nm and an emission wavelength of 430–470 nm. Signals for the detector were estimated by peak height using a Cole-Parmer model 8384-32 chart recorder.

Concentrations of DA and other amines were determined by HPLC with electrochemical detection as previously described (Sloley and Orikasa, 1988; Sloley et al., 1991).

As single fish pituitaries and olfactory bulbs were difficult to weigh accurately, estimates of amino acid and DA concentrations were based on the amount of protein present. Protein content determinations were performed using the Bio-Rad protein assay method using bovine serum albumin as the standard (Bradford, 1976).

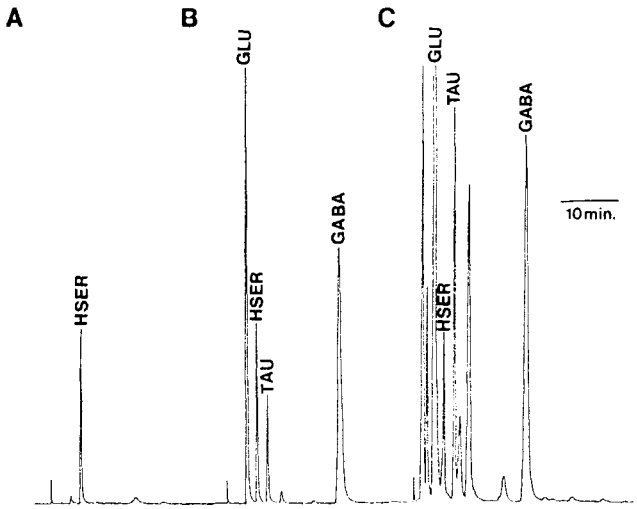

FIG. 1. Sample chromatograms of *o*-phthalaldehyde derivatives of glutamate (GLU), homoserine (HSER), taurine (TAU), and GABA in standards and extract of whole fish brain. **A:** Derivatized extraction solution containing 12.5 ng of homoserine. **B:** Derivatized standard of 12.5 ng of glutamate, homoserine, taurine, and GABA. **C:** Derivatized ethanol extract of whole fish brain with homoserine added as the internal standard, representing ~10 μg wet weight of tissue.

TABLE 1. *Distribution of GABA, glutamate, and taurine in brain and pituitary of sexually recrudescent male and female goldfish*

Tissue (n)	ng/μg of protein		
	GABA	Glutamate	Taurine
Telencephalon (8)	2.16 ± 0.12	11.1 ± 0.8	22.1 ± 1.9
Hypothalamus (8)	1.40 ± 0.20	6.69 ± 0.93	12.2 ± 1.4
Cerebellum (8)	0.99 ± 0.07	7.89 ± 0.65	11.5 ± 0.9
Optic tectum (8)	3.08 ± 0.11	10.5 ± 0.6	17.0 ± 1.0
Posterior medulla (8)	1.74 ± 0.28	7.65 ± 1.05	10.9 ± 1.3
Pituitary (8)	0.68 ± 0.04	4.48 ± 0.61	20.0 ± 1.9
Olfactory bulbs (8)	1.54 ± 0.10	3.78 ± 0.30	10.2 ± 0.9

Data are mean ± SEM values based on n determinations.

Statistical analyses

Data are expressed as mean ± SEM values. Differences between means were examined by analysis of variance. Means were considered statistically different if $p < 0.05$.

RESULTS

Figure 1 illustrates chromatograms derived from the extraction solution, standards, and extract of goldfish brain. The internal standard (homoserine), GABA, glutamate, and taurine were well resolved from other fluorescent derivatives found in extracts of fish brain. Other amino acid derivatives observed, but not quantified, included tyrosine and phenylalanine. Recovery of the internal standard from tissue extractions was 90%.

The concentrations of GABA, taurine, and glutamate in various regions of the goldfish brain and pituitary are shown in Table 1. GABA and glutamate were found predominantly in the optic tectum and telencephalon. The highest concentrations of taurine were found in the telencephalon and pituitary. As can be noted in some tables, e.g., Tables 2–4, concentrations of amino acid neurotransmitters varied somewhat between experiments. For example, in the pituitary, taurine concentrations ranged from 4 to 20 ng/μg of protein. These variations appeared to derive from age or seasonal differences in fish used.

Short-term anesthesia with tricaine methanesulfonate had no effects on GABA, glutamate, or taurine concentrations in the goldfish hypothalamus, telencephalon, pituitary, olfactory bulbs, cerebellum, optic tectum, and posterior medulla (data not shown).

Permitting pituitary tissue to remain at room temperature before freezing did not result in alterations in GABA concentrations (Fig. 2A). However, GABA concentrations in the hypothalamus (Fig. 2B) and telencephalon (Fig. 2C) increased with the time the tissue was permitted to remain at room temperature before freezing. Neither glutamate nor taurine concentrations were significantly dependent on the time tissues were permitted to remain at room temperature before freezing (data not shown).

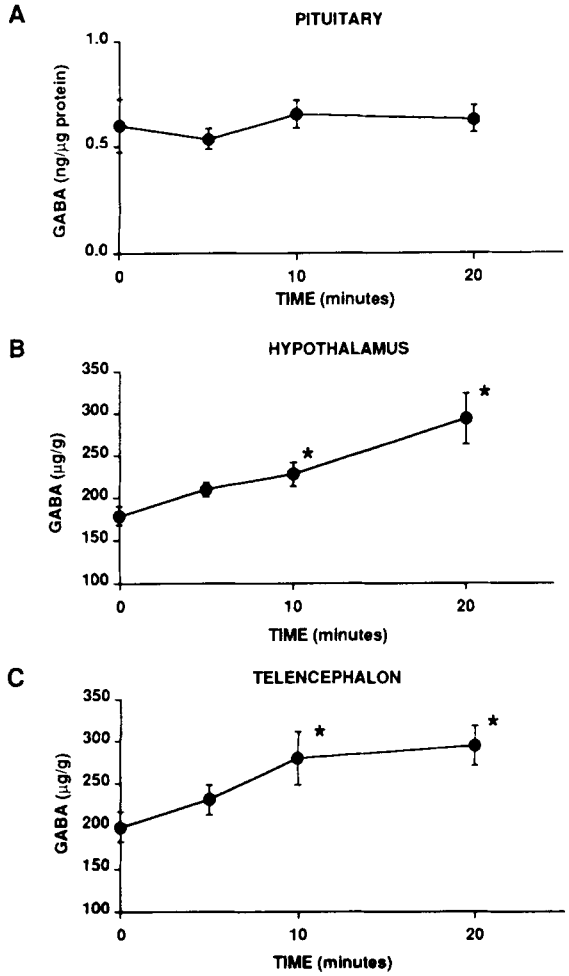

FIG. 2. Postmortem changes in the concentrations of GABA in (**A**) pituitary, (**B**) hypothalamus, and (**C**) telencephalon of goldfish. Fish were anesthetized, and the pituitary and brain regions were dissected out and held in polypropylene microtubes at room temperature for various times until freezing on dry ice. Data are mean ± SEM (bars) values (n = 12). *Significantly different from time = 0 min ($p < 0.05$).

Intraperitoneal injection of the L-glutamic acid decarboxylase inhibitor 3-MPA (McGeer and McGeer, 1989) (50 μg/g, 2 h) resulted in a significant reduction in GABA, but not glutamate or taurine, concentrations in the goldfish pituitary; pituitary GABA concentrations declined from 0.72 ± 0.05 (n = 7) to 0.54 ± 0.08 ng/μg of protein (n = 5). In the telencephalon, 3-MPA caused a reduction in taurine concentrations [decline from 343 ± 34 (n = 7) to 235 ± 36 μg/g (n = 5); $p < 0.05$]; however, GABA and glutamate levels remained unchanged.

Intraperitoneal injection of γ-vinyl GABA into goldfish undergoing gonadal recrudescence resulted in a dose-dependent increase in GABA concentrations in the pituitary (Table 2), hypothalamus (Table 3), and telencephalon (data not shown) lasting at least 48 h. Taurine concentrations in the pituitary (Table 2) but not in the hypothalamus (Table 3) or telencephalon (data not shown) were elevated by γ-vinyl GABA administration. Serum GTH concentrations were also elevated by γ-vinyl GABA (Table 2). DA, norepinephrine, 5-HT, and 5-hydroxyindoleacetic acid concentrations were not altered in pituitary (Table 2), hypothalamus (Table 3), or telencephalon (data not shown) by γ-vinyl GABA treatment. Intracerebroventricular injection of GABA (200 μg in 2 μl, 30 min) significantly elevated serum GTH concentrations from 16.3 ± 2.1 (n = 12) to 65.9 ± 11.2 ng/ml (n = 12; $p < 0.05$).

Intraperitoneal injection of taurine caused a significant increase in serum GTH concentrations at 30 min after administration (Fig. 3 and Table 4). This effect was also seen after injection of the taurine precursor hypotaurine and the structurally related amino acid β-alanine but not cysteic acid, isetheonic acid, lysine, or glutamate (Fig. 3). Intracerebroventricular administration of taurine also elevated serum GTH concentrations from 16.3 ± 2.1 (n = 12) to 28.9 ± 6.4 ng/ml (n = 12; $p < 0.05$).

Pretreatment with α-MPT, which reduced concentrations of brain DA and norepinephrine (data not shown) and pituitary DA (Table 4) and potentiated the serum GTH response to a GnRH analogue (Peter et al., 1986), also potentiated the GTH response to taurine (Table 4).

Injection of MSG into sexually recrudescent goldfish resulted in a significant increase in pituitary glutamate and taurine concentrations and a decrease in pituitary DA concentrations at 24 h after injection (Table 5). This was followed by a decrease in pituitary GABA, glutamate, and taurine concentrations at 72 h after injection; pituitary DA concentrations remained significantly reduced 72 h after MSG injection. Concentrations of GABA, glutamate, taurine, and DA recovered by 96 h after injection. In the hypothalamus the only significant result of MSG injection was a decrease in glutamate concentrations from 1.29 ± 0.04 (n = 12) to 1.04 ± 0.04 mg/g (n = 12; $p < 0.05$) 72 h after injection. No MSG-dependent changes in telencephalon amino acid neurotransmitter concentrations were demonstrated (data not shown).

Serum GTH concentrations were elevated from 16.8 ± 2.7 (n = 12) to 31.0 ± 6.0 ng/ml (n = 12; $p < 0.05$) at 24 h, but not at other sample times, after MSG administration. Fish treated with MSG for 48 h before the administration of sGnRHa showed a potentiated serum GTH response (Fig. 4). More specifically, fish in midstages of ovarian recrudescence (GSI = 5.7 ± 1.2; n = 10) were not responsive to sGnRHa (0.1 μg/g, 6 h) alone; however, fish pretreated with MSG showed a marked elevation of serum GTH concentration following sGnRHa injection (Fig. 4A). Fish in the late stages of ovarian recrudescence (GSI = 9.9 ± 1.3; n = 10) were responsive to sGnRHa (0.01 μg/g, 6 h) alone; fish treated with MSG also showed a

TABLE 2. *Effects of intraperitoneal injection of γ-vinyl GABA on serum GTH levels and GABA, glutamate, taurine, DA, and 5-HT concentrations in the pituitary of sexually recrudescent goldfish*

Treatment	GTH (ng/ml)	GABA (ng/μg of protein)	Glutamate (ng/μg of protein)	Taurine (ng/μg of protein)	DA (pg/μg of protein)	5-HT (pg/μg of protein)
Saline (24 h)	16.0 ± 2.5	1.19 ± 0.10	3.19 ± 0.20	4.27 ± 0.66	1.64 ± 0.20	0.29 ± 0.04
γ-Vinyl GABA						
100 μg/g, 24 h	23.7 ± 5.2	5.82 ± 0.38[a]	3.41 ± 0.30	6.70 ± 0.67[a]	1.58 ± 0.17	0.19 ± 0.03
300 μg/g, 24 h	74.2 ± 11.4[a]	6.23 ± 0.45[a]	3.23 ± 0.26	5.88 ± 0.50[a]	1.84 ± 0.18	0.25 ± 0.04
Saline (48 h)	15.1 ± 1.8	1.68 ± 0.26	3.43 ± 0.27	5.56 ± 0.62	1.80 ± 0.27	0.25 ± 0.03
γ-Vinyl GABA						
(100 μg/g, 48 h)	29.1 ± 5.3[a]	15.3 ± 1.4[a]	3.90 ± 0.27	6.41 ± 0.49	1.44 ± 0.21	0.32 ± 0.09

Norepinephrine and 5-hydroxyindoleacetic acid were not detectable in the pituitary, similar to previous results (Sloley et al., 1991). Data are mean ± SEM values (n = 6–8).

[a] Significantly different from time-matched controls ($p < 0.05$). The values for GABA have also been presented elsewhere (Kah et al., 1992).

potentiation of the serum GTH response to sGnRHa (Fig. 4B).

DISCUSSION

The present study indicates that, like other vertebrates, fish possess high concentrations of GABA, glutamate, and taurine in the brain and pituitary. Pharmacological manipulations of both GABA and taurine suggest that these compounds have an important role in the regulation of GTH secretion.

As in mammals (Alderman and Schellenberger, 1974), postmortem effects on goldfish brain GABA concentrations were evident. In both the hypothalamus and telencephalon, GABA concentrations increase with time until freezing of the tissue. This increase is relatively slow, and immediate freezing of dissected tissues on dry ice prevents significant accumulation of GABA. Killing by means of microwave irradiation has been suggested as a means of avoiding postmortem elevations of GABA levels in mammalian nervous tissue (Alderman and Scallenberger, 1974). This is impractical with fish, and rapid freezing of dissected tissue appears to be sufficient to avoid irregularities in GABA content measurement. No postmortem effects on GABA concentrations were demonstrated in the pituitary. This may indicate that the GABAergic fibers located in the pituitary have a lesser capacity to synthesize GABA in comparison with the CNS. Neither glutamate nor taurine demonstrated postmortem elevations in level, indicating that postmortem alterations in the concentrations of these compounds are minimal.

As anesthesia with tricaine methanesulfonate did not alter neurotransmitter amino acid concentrations in any brain region, this anesthetic was considered to have minimal impact on sampling procedures. It must be noted that all exposures to anesthetic before sampling were held to a minimum and that no samples were removed from fish that had been exposed to tricaine methanesulfonate for >3 min.

After validation of the sampling and measurement of GABA, glutamate, and taurine levels in fish nervous tissue and pituitary, tissue concentrations and distribution of these compounds were examined. GABA, glutamate, and taurine are found in all brain

TABLE 3. *Effect of intraperitoneal injection of γ-vinyl GABA on GABA, glutamate, taurine, norepinephrine (NE), DA, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the hypothalamus of sexually recrudescent goldfish*

Treatment	n	GABA (μg/g)	Glutamate (mg/g)	Taurine (μg/g)	NE (ng/g)	DA (ng/g)	5-HT (ng/g)	5-HIAA (ng/g)
Saline (24 h)	7	156 ± 10	1.54 ± 0.12	409 ± 45	1,439 ± 234	512 ± 64	637 ± 86	75 ± 13
γ-Vinyl GABA								
100 μg/g, 24 h	7	573 ± 27[a]	1.44 ± 0.03	424 ± 26	1,464 ± 170	494 ± 29	634 ± 63	85 ± 6
300 μg/g, 24 h	7	822 ± 39[b]	1.55 ± 0.07	401 ± 22	1,497 ± 135	542 ± 41	611 ± 32	68 ± 2
Saline (48 h)	8	136 ± 9	1.42 ± 0.11	376 ± 20	1,495 ± 70	649 ± 72	781 ± 45	71 ± 11
γ-Vinyl GABA								
(100 μg/g, 48 h)	8	1021 ± 96[a]	1.53 ± 0.13	405 ± 23	1,748 ± 213	575 ± 16	793 ± 36	100 ± 7

Data are mean ± SEM values based on n determinations.

[a] Significantly different from time-matched controls ($p < 0.05$).

[b] Significantly different from time-matched controls and 100 μg/g of γ-vinyl GABA, 24 h ($p < 0.05$).

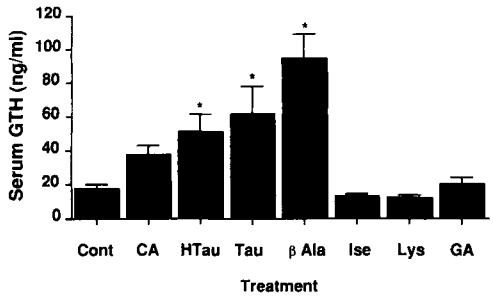

FIG. 3. Effects of intraperitoneal injection of saline (Cont), L-cysteic acid (CA), hypotaurine (HTau), taurine (Tau), β-alanine (β Ala), isetheonic acid (Ise), L-lysine (Lys), and L-glutamic acid (GA) at doses of 1.0 mg/g on serum GTH concentrations 30 min later in sexually recrudescent female goldfish. Data are mean ± SEM (bars) values (n = 10–12). *Significantly different from controls ($p < 0.05$).

regions and the pituitary, and, as might be expected, there is a regional distribution of the amino acid neurotransmitters within these tissues.

The concentrations of these amino acid neurotransmitters determined in the goldfish approximate those observed in other vertebrates (Sturman et al., 1978) and fish (Nilsson, 1990). We did find, however, that amino acid neurotransmitter concentrations (especially that of taurine) could vary substantially from stock to stock or at various times of the year. It has been demonstrated that free amino acid concentrations in nervous tissue can vary greatly in the nervous tissue of fish and other vertebrates. For example, older, larger pike have brain tryptophan concentrations that are much lower than those of younger, smaller pike (Sloley and Rehnberg, 1988). In mammals, taurine levels in the brain are very dependent on the age of the animal (Sturman et al., 1978), with levels in rat brain falling from 17 μmol/g at birth to 4.24 μmol/g by maturation. It is likely that because goldfish are seasonal breeders, amino acid concentrations in the brain and pituitary will alter with the age of the stock and be reflected in changes in concentration throughout the year.

In goldfish, GABA is concentrated within the optic tectum and olfactory bulbs, and its level is lowest in the cerebellum and pituitary. These observations, in part, confirm the immunohistochemical studies of Martinoli et al. (1990), in which strong GABA immunoreactivity was described in the olfactory bulbs. The occurrence of GABA in the goldfish pituitary also confirms the immunohistochemical demonstration of direct GABA innervation of this organ (Kah et al., 1987). The presence of high concentrations of GABA in the optic tectum and olfactory bulbs suggests that, in fish, GABA may play a role in the processing or regulating of sensory information. In contrast to GABA, glutamate concentrations are fairly evenly distributed throughout the brain of the goldfish, although lower levels are observed in the pituitary and olfactory bulbs.

Taurine is found in highest concentrations in the telencephalon, optic tectum, and pituitary, paralleling results of studies on mammals (Guidotti, 1978). The extremely high concentrations of taurine in the pituitary strongly suggest a role for this compound in the regulation of pituitary function.

Inhibitors of the synthesis and degradation of GABA were investigated both to confirm the accuracy of the analytical technique and to investigate the involvement of GABA in pituitary function. 3-MPA, an inhibitor of L-glutamic acid decarboxylase (McGeer and McGeer, 1989), significantly reduced GABA concentrations in the pituitary but not in the brain or telencephalon. Taurine concentrations in the telencephalon, but not pituitary or hypothalamus, were also reduced by 3-MPA. These results indicate that although 3-MPA might inhibit goldfish L-glutamic acid decarboxylase activity, the effects are not selective to the GABAergic system and may be subject to regional differences. Because of these problems with the effects of 3-MPA, we discontinued investigation of this compound.

Injection of γ-vinyl GABA resulted in a potent and relatively selective increase in GABA concentrations in the goldfish brain and pituitary lasting at least 48 h. This drug has been demonstrated to inhibit irrevers-

TABLE 4. *Effect of intraperitoneal injection of taurine (1 mg/g, 30 or 60 min) on serum GTH and pituitary DA concentrations in sexually recrudescent goldfish with or without pretreatment with α-MPT (200 μg/g, 5 days)*

Treatment	GTH (ng/ml) after taurine injection		Pituitary DA (pg/μg of protein)
	30 min	60 min	
Saline	11.6 ± 1.6 (12)	9.9 ± 1.3 (12)	5.02 ± 0.54 (7)
Taurine	35.3 ± 7.7 (10)[a]	20.9 ± 3.2 (12)[a]	ND
α-MPT	12.0 ± 1.5 (12)	13.3 ± 2.8 (12)	1.53 ± 0.15 (7)[a]
α-MPT and taurine	28.3 ± 7.2 (9)[a]	37.2 ± 6.6 (11)[b]	ND

Data are mean ± SEM values based on n determinations. ND, not determined.
[a] Significantly different from controls ($p < 0.05$).
[b] Significantly different from controls and taurine-injected animals ($p < 0.05$) at 30 min.

TABLE 5. *Effects of intraperitoneal injection of MSG (2.5 mg/g) at 24, 72, and 96 h after injection on GABA, glutamate, taurine, and DA concentrations in the pituitary of sexually recrudescent goldfish*

Treatment	GABA (ng/μg of protein)	Glutamate (ng/μg of protein)	Taurine (ng/μg of protein)	DA (pg/μg of protein)
Saline 24 h	0.62 ± 0.09	5.12 ± 0.64	14.4 ± 0.8	2.18 ± 0.14
MSG 24 h	0.76 ± 0.04	10.2 ± 1.00[a]	21.6 ± 1.2[a]	1.68 ± 0.11[a]
Saline 72 h	0.60 ± 0.03	7.91 ± 0.53	16.4 ± 0.7	1.92 ± 0.13
MSG 72 h	0.46 ± 0.03[a]	5.29 ± 0.49[a]	13.7 ± 0.8[a]	1.45 ± 0.16[a]
Saline 96 h	0.49 ± 0.03	6.36 ± 0.55	11.5 ± 0.6	2.84 ± 0.26
MSG 96 h	0.52 ± 0.03	6.46 ± 0.70	13.1 ± 0.8	2.36 ± 0.19

Data are mean ± SEM values (n = 12).
[a] Significantly different from time matched controls ($p < 0.05$).

ibly GABA transaminase in rats (Brustle et al., 1988), resulting in an elevation of GABA levels. γ-Vinyl GABA appears to have the same effects in goldfish, and the effect is observed on both sides of the blood–brain barrier. The effects of γ-vinyl GABA are reasonably selective, with no effects demonstrated on DA, norepinephrine, 5-HT, or glutamate concentrations in hypothalamus or pituitary and only a transient increase of taurine concentrations in the telencephalon. In addition to greatly elevating GABA concentrations, injections of γ-vinyl GABA caused highly elevated serum GTH levels in recrudescent fish, suggesting a role for GABA in the regulation of release of this hormone. GABA is generally believed to be involved in the inhibitory control of luteinizing hormone secretion in mammals (McCann and Rettori, 1988). However, in rats, GABA has also been demonstrated to elevate levels of serum luteinizing hormone (Vijayan and McCann, 1978*a*) and growth hormone (Vijayan and McCann, 1978*b*) and to lower levels of serum thyrotropin (Vijayan and McCann, 1978*b*) and prolactin (Schally et al., 1977). These effects have been suggested to be due to the ability of GABA to modulate DA activity in the brain and pituitary (reviewed by McCann and Rettori, 1988), although this has not been established for all of the pituitary hormones affected. The present data do not clearly support the proposal that GABA modulates DA release in the fish pituitary and thereby affects GTH release; injection of γ-vinyl GABA caused increased pituitary and brain GABA levels and increased serum GTH concentrations without altering pituitary or brain DA concentrations. In addition, direct effects of GABA on GnRH release (Kah et al., 1992) may be involved in the responses to γ-vinyl GABA.

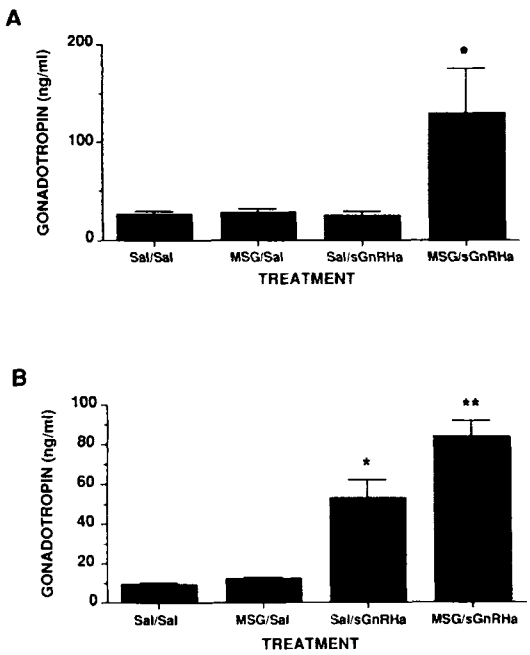

FIG. 4. Effects of MSG pretreatment on the serum GTH response to sGnRHa. **A:** MSG (2.5 mg/g) potentiates GTH release in fish that are unresponsive (GSI = 5.37 ± 1.15; n = 10) to 0.1 μg/g of sGnRHa alone. **B:** MSG also potentiates GTH release in fish that are responsive to 0.01 μg/g of sGnRHa (GSI = 9.86 ± 1.25; n = 10). *Significantly different from controls ($p < 0.05$). **Significantly different from controls and sGnRHa-injected animals ($p < 0.05$). Sal, saline (control).

Both intraperitoneal and intracerebroventricular injection of taurine caused an increase in serum GTH levels in goldfish undergoing gonadal recrudescence. Taurine has been suggested to parallel GABA in its inhibitory effects on dopaminergic systems (Ahtee and Vahala, 1985) and as such might be expected to exert similar effects on GTH release in goldfish. If taurine action is similar to that of GABA, the taurine-induced elevation of GTH levels in fish would be consistent with the elevated luteinizing hormone concentrations observed after GABA treatment in rats (Vijayan and McCann, 1978*a*). With respect to GTH release in fish, taurine injection elevates serum GTH levels in a manner similar to that described after injection of GABA or elevation of GABA levels due to inhibition of GABA transaminase (this study; Kah et al., 1992). However, taurine stimulation of GTH release in fish is in contrast to rats, where taurine injection has no effect on LH release but blocks the *N*-

methyl-D-aspartate-evoked release of LH (Price et al., 1978).

Pretreatment with α-MPT has been demonstrated to potentiate the GTH response to GnRH analogues in goldfish (Peter et al., 1986) and Chinese loach (Lin et al., 1986), presumably by attenuating the inhibitory tone of DA on GTH release. The results presented here support the idea that taurine and DA modulate GTH release. The effect of taurine on GTH release appears to be dependent on the inhibitory tone of DA in the pituitary. Whether this effect of taurine is through modulation of DA, GnRH, or other neuroendocrine factors remains to be determined.

MSG has been demonstrated to cause selective lesions in the nucleus lateral tuberis and the nucleus preopticus periventricularis of the goldfish forebrain (Kah et al., 1983) and is known to affect sexual maturation (MacDonald and Wilkinson, 1990) and the luteinizing hormone response to DA agonists (Rose and Weick, 1986) in rats. The cell attrition resulting from MSG has been proposed to be the result of overstimulation of glutamate-responsive cells (Olney, 1988). Our results indicate that, rather than having a specific effect on any one of the aminergic or amino acid neurotransmitters in the pituitary, MSG causes a transient elevation of glutamate content followed by a general depletion of glutamate, GABA, taurine, and DA. This does not appear to be due to specific attrition of neurons containing these neurotransmitters, as the effect is transient, but may result from the general damage to areas through which these neurons travel. The elevation of GTH levels may ultimately result from a transient loss of inhibitory DA tone on the gonadotrophs through either loss of DA or loss of ability of these inhibitory neurons to maintain normal activity. The potentiation of the serum GTH response to sGnRHa in MSG-treated fish is consistent with this proposal, as inhibition of dopaminergic systems by DA antagonists or DA synthesis inhibitors also potentiates the serum GTH response to GnRH or agonistic GnRH analogues (Peter et al., 1986).

In conclusion, GABA, glutamate, and taurine are found throughout the goldfish brain and pituitary. Taurine concentrations are particularly high in the pituitary. GABA and taurine have similar stimulatory effects on GTH secretion. The results indicate that stimulatory effects of GABA and taurine may be, in part, due to an interaction with the DA system, which has inhibitory effects on GnRH and GTH release in the goldfish.

Acknowledgment: This research was supported by grant A6371 from the Natural Sciences and Engineering Research Council of Canada to R. E. Peter, an Alberta Heritage Foundation for Medical Research Fellowship to B. D. Sloley, and an Alberta Heritage Foundation for Medical Research Studentship to V. L. Trudeau. A CNRS-MRC Exchange Program Grant to O. Kah is gratefully acknowledged.

REFERENCES

Ahtee L. and Vahala M.-L. (1985) Taurine and its derivatives alter brain dopamine metabolism similarly to GABA in mice and rats. *Prog. Clin. Biol. Res.* **171,** 331–341.

Alderman J. L. and Schellenberger M. K. (1974) γ-Aminobutyric acid (GABA) in the rat brain: re-evaluation of sampling procedures and the post-mortem increase. *J. Neurochem.* **22,** 937–943.

Anden N.-E. and Stock G. (1973) Inhibitory effect of gammahydroxybutyric acid and gammaaminobutyric acid on the dopamine cells in the substantia nigra. *Naunyn Schmiedbergs Arch. Pharmacol.* **279,** 89–92.

Biswas B. and Carlsson A. (1977) The effect of intracerebroventricularly administered GABA on brain monoamine metabolism. *Naunyn Schmiedbergs Arch. Pharmacol.* **299,** 47–51.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Brustle O., Pilgrim Ch., Gaymann W., and Reisert I. (1988) Abundant GABAergic innervation of rat posterior pituitary revealed by inhibition of GABA-transaminase. *Cell Tissue Res.* **251,** 59–64.

Chang J. P. and Peter R. E. (1983) Effects of dopamine on gonadotropin release in female goldfish, *Carassius auratus*. *Neuroendocrinology* **36,** 351–357.

Chang J. P., Yu K. L., Wong A. O. L., and Peter R. E. (1990) Differential actions of dopamine receptor subtypes on gonadotropin and growth hormone release *in vitro* in goldfish. *Neuroendocrinology* **51,** 664–674.

Chang J. P., Van Goor F., and Acharya S. (1991) Influences of norepinephrine, and adrenergic agonists and antagonists on gonadotropin secretion from dispersed pituitary cells of goldfish, *Carassius auratus*. *Neuroendocrinology* **51,** 202–210.

Guidotti A. (1978) Role of taurine in brain function, in *Taurine and Neurological Disorders* (Barbeau A. and Huxtable R. J., eds), pp. 237–248. Raven Press, New York.

Kah O., Peter R. E., Dubourg P., and Cook H. (1983) Effects of monosodium L-glutamate on pituitary innervation in goldfish, *Carassius auratus*. *Gen. Comp. Endocrinol.* **51,** 338–346.

Kah O., Dubourg P., Martinoli M. G., Rabhi M., Gonnet F., Geffard M., and Calas A. (1987) Central GABAergic innervation of the pituitary in goldfish: a radioautographic and immunocytochemical study at the electron microscope level. *Gen. Comp. Endocrinol.* **67,** 324–332.

Kah O., Trudeau V. L., Sloley B. D., Chang J. P., Dubourg P., Yu K. L., and Peter R. E. (1992) Influence of GABA on gonadotrophin release in the goldfish. *Neuroendocrinology* (in press).

Kontro P. (1987) Interactions of taurine and dopamine in the striatum. *Adv. Exp. Med. Biol.* **217,** 347–355.

Lin H. R., Peng C., Van Der Kraak G., Peter R. E., and Breton B. (1986) Effects of [D-Ala^6, Pro^9-NEt]-LHRH and catecholaminergic drugs on gonadotropin secretion and ovulation in the Chinese loach (*Paramisgurnus dabryanus*). *Gen. Comp. Endocrinol.* **64,** 389–395.

MacDonald M. C. and Wilkinson M. (1990) Peripubertal treatment with *N*-methyl-D-aspartic acid or neonatally with monosodium glutamate accelerates sexual maturation in female rats, an effect reversed by MK-801. *Neuroendocrinology* **52,** 143–149.

Martinoli M. G., Dubourg P., Geffard M., Calas A., and Kah O. (1990) Distribution of GABA-immunoreactive neurones in the forebrain of the goldfish. *Cell Tissue Res.* **260,** 77–84.

McCann S. M. and Rettori V. (1988) The role of gamma amino butyric acid (GABA) in the control of anterior pituitary hormone secretion, in *GABA and Benzodiazepine Receptors, Vol. 1* (Squires R. F., ed), pp. 123–134. CRC Press, Boca Raton, Florida.

McCann S. M., Vijayan E., Negro-Vilar E., Mizunuma H., and Mangat H. (1984) Gamma-aminobutyric acid (GABA), a modulator of anterior pituitary hormone secretion by hypotha-

lamic and pituitary action. *Psychoneuroendocrinology* **9,** 97–106.

McGeer P. L. and McGeer E. G. (1989) Amino acid neurotransmitters, in *Basic Neurochemistry*, 4th ed. (Siegel G., Agranoff B., Albers R. W., and Molinoff P., eds), pp. 311–332. Raven Press, New York.

Nilsson G. E. (1990) Long-term anoxia in crucian carp: changes in the levels of amino acid and monoamine neurotransmitters in the brain, catecholamines in chromaffin tissue, and liver glycogen. *J. Exp. Biol.* **150,** 295–320.

Olney J. W. (1988) Revelations in excitotoxicology: what next? *Neurol. Neurobiol.* **46,** 589–596.

Peng C., Huang Y.-P., and Peter R. E. (1990) Neuropeptide Y stimulates growth hormone and gonadotropin release from the goldfish pituitary in vitro. *Neuroendocrinology* **52,** 28–34.

Peter R. E., Nahorniak C. S., Chang J. P., and Crim L. W. (1984) Gonadotropin release from pars distalis of goldfish, *Carassius auratus*, transplanted beside the brain or into the brain ventricles: additional evidence for gonadotropin-release-inhibitory factor. *Gen. Comp. Endocrinol.* **55,** 337–346.

Peter R. E., Chang J. P., Nahorniak C. S., Omeljaniuk R. J., Sokolowska M., Shih S. H., and Billard R. (1986) Interactions of catecholamines and GnRH in regulation of gonadotropin in teleost fish. *Recent Prog. Horm. Res.* **42,** 513–548.

Peter R. E., Yu K. L., Marchant T. A., and Rosenblum P. M. (1990) Direct neural regulation of teleost adenohypophysis. *J. Exp. Zool.* **4** (Suppl), 84–89.

Price M. T., Olney J. W., Mitchell M. V., Fuller T., and Cicero T. J. (1978) Luteinizing hormone releasing action of *N*-methyl aspartate is blocked by GABA or taurine but not by dopamine antagonists. *Brain Res.* **158,** 461–465.

Rose P. A. and Weick R. F. (1986) Hyperresponsiveness to the inhibitory action of dopamine agonists on luteinizing hormone secretion in the monosodium-L-glutamate treated, orchidectomized rat. *Neuroendocrinology* **44,** 95–101.

Rosenblum P. M. and Peter R. E. (1989) Evidence for the involvement of endogenous opioids in the regulation of gonadotropin secretion in male goldfish. *Gen. Comp. Endocrinol.* **73,** 21–27.

Schally A. V., Redding T. W., Arimura A., Dupont A., and Linthicum G. L. (1977) Isolation of gamma-aminobutyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF) activity "in vivo" and "in vitro." *Endocrinology* **100,** 681–691.

Sloley B. D. and Orikasa S. (1988) Selective depletion of dopamine, octopamine, and 5-hydroxytryptamine in the nervous tissue of the cockroach (*Periplaneta americana*). *J. Neurochem.* **51,** 535–541.

Sloley B. D. and Rehnberg B. G. (1988) Noradrenaline, dopamine, 5-hydroxytryptamine and tryptophan concentrations in the brains of four cohabiting species of fish. *Comp. Biochem. Physiol.* [*C*] **89,** 197–199.

Sloley B. D., Trudeau V. L., Dulka J. G., and Peter R. E. (1991) Selective depletion of dopamine in the goldfish pituitary caused by domperidone. *Can. J. Physiol. Pharmacol.* **69,** 776–781.

Somoza G. M. and Peter R. E. (1991) Effects of serotonin on gonadotropin and growth hormone release from *in vitro* perfused goldfish pituitary fragments. *Gen. Comp. Endocrinol.* **82,** 103–110.

Somoza G. M., Yu K. L., and Peter R. E. (1988) Serotonin stimulates gonadotropin release in female and male goldfish, *Carassius auratus* L. *Gen. Comp. Endocrinol.* **72,** 374–382.

Sturman J. A., Rassin D. K., and Gaull G. E. (1978) Taurine in the development of the central nervous system, in *Taurine and Neurological Disorders* (Barbeau A. and Huxtable R. J., eds), pp. 49–71. Raven Press, New York.

Tappaz M. L., Wassef M., Oertel W. H., Paut L., and Pujol J. F. (1983) Light and electron-microscopic immunocytochemistry of glutamic acid decarboxylase (GAD) in the basal hypothalamus: morphological evidence for neuroendocrine gamma-aminobutyrate (GABA). *Neuroscience* **9,** 271–283.

Vijayan E. and McCann S. M. (1978*a*) The effects of intraventricular injection of γ-aminobutyric acid (GABA) on prolactin and gonadotropin release in conscious female rats. *Brain Res.* **155,** 35–43.

Vijayan E. and McCann S. M. (1978*b*) The effects of intraventricular injection of γ-aminobutyric acid (GABA) on plasma growth hormone (GH) and thyrotropin (TSH) release in conscious ovariectomized rats. *Endocrinology* **103,** 1888–1893.

Yu K. L. and Peter R. E. (1990) Dopaminergic regulation of brain gonadotropin-releasing hormone in male goldfish during spawning behaviour. *Neuroendocrinology* **52,** 276–283.

Yu K. L., Nahorniak C. S., Peter R. E., Corrigan A., Rivier J. E., and Vale W. W. (1987) Brain distribution of radioimmunoassayable gonadotropin-releasing hormone in female goldfish: seasonal variation and periovulatory changes. *Gen. Comp. Endocrinol.* **67,** 234–246.

Yu K. L., Rosenblum P. M., and Peter R. E. (1991) *In vitro* release of gonadotropin-releasing hormone from the brain preoptic-anterior hypothalamic region and pituitary of female goldfish. *Gen. Comp. Endocrinol.* **81,** 256–267.