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REVIEW

Control of Prolactin and Growth Hormone Secretion in Teleost Fishes

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INTRODUCTION

In teleost fish, hypothalamic fibers terminate in proximity to adenohypophysial (AH) cells of the pituitary, thereby circumventing a functional median eminence and a hypophysial portal system, structures considered to be both ancient and conservative in vertebrate evolution (see [1–3]). Thus, unlike the situation in most other vertebrates, teleosts have a more or less direct neural control of pituitary function. Although claims for a median eminence-portal system have been made for some teleostean species (see [4, 5]), the existence of such a system is generally considered absent from teleosts [2, 3, 6–8].

Two general classes of hypothalamic nerve fibers innervate the pituitary of all teleosts investigated to date: type A fibers, containing "elementary neurosecretory granules" (115–170 nm granule diameter) and generally considered to be peptidergic, and type B fibers, containing "large granulated vesicles" (45–95 nm granule diameter) and generally considered to be aminergic. A possible third type of hypothalamic fiber, however, is observed in *Oreochromis mossambicus* (tilapia) [9]; these "type C" fibers are similar to type A fibers, but are characterized by granules of intermediate size (90–145 nm granule diameter) with limiting membranes usually separated from the granule core. Type A fibers, because they are commonly observed leading into the neurohypophysis (NH) and pars intermedia (PI) from the preoptic nuclei (PON), are believed to be concerned primarily with the secretion of neurohypophysial octapeptides and the control of the PI, whereas type B fibers, because they are commonly observed emanating from the nucleus lateralis tuberis (NLT) and ending in synaptic contact with the secretory cells of the pars distalis (PD), are believed to be more closely involved in the control of the PD [2, 6]. Type C fibers of tilapia are observed regularly in the hypothalamus and neurohypophysis, but no physiological function can be attributed to these fibers on the basis of morphological criteria alone [9]. More recently, however, physiological evidence of important peptidergic control of the PD has modified these early views; thus, type A (and possibly in tilapia, type C) control of PD function is considered in this review.

The focus of this review is the control of prolactin (PRL) and growth hormone (GH) secretion in teleosts, with primary emphasis on the tilapia; therefore, the hypothalamus and other parts of the brain will not be extensively discussed except as they relate directly to the material presented herein (for earlier reviews and articles of neural control of teleost adenohypophysial function, see [2–4, 6, 10–13]). Briefly, many different hypothalamic nuclei exist in teleosts, a number of which may be important in the control of AH function. Two nuclei previously mentioned have been particularly well studied: the PON and the NLT. The PON occurs in tilapia as layers of neurons alternating with layers of axonal processes

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lying above the optic chiasma; in general, the cells of the PON are type A and can be divided into two groups, a ventral pars parvocellularis and a dorsal pars magnocellularis. Axonal processes from the PON converge mid-ventrally, progress along the NLT region into the pituitary, and become a part of the NH [2, 11, 13, 14]. The NLT, on the other hand, is usually present in teleosts as a pair of structures lying adjacent to the anterior and lateral regions of the base of the infundibular stalk from which, most commonly, type B fibers run in a main tract alongside type A (PON) fibers into the NH [1, 2, 10, 11, 14].

ANATOMICAL ASPECTS

The degree of directness of hypothalamic innervation of pituitary cells varies in the different regions of the pituitary, as well as among teleosts. In the case of the tilapia rostral pars distalis (RPD), hypothalamic fibers do not leave the NH but instead terminate on an adjacent basement membrane (see [15, 16]). Similar observations have been made on *Fundulus heteroclitus* [17] and on *Carassius auratus* [18, 19]. As a consequence of this anatomical arrangement, chemical information from the brain must pass through the basement membrane, a layer of stellate cell processes, and also the adrenocorticotropic hormone (ACTH) cells surrounding the neurohypophysial processes [15] (Fig. 1). Because of their direct contact with PRL cells, stellate cells and their processes may be involved directly in information transfer; perhaps stellate cells modulate movement of neurohormonal factors from the neurohypophysial nerve endings to the PRL cells located several cell layers away. The stellate cells may be equivalent to the tanycytes that link the third ventricle with the NH [20]. The role of the intervening layer of ACTH cells in information transfer, if any, is unknown, but a paracrine role for one or more proopiomelanocorticotropin products is possible (Fig. 1).

The anatomical basis for control of GH secre-

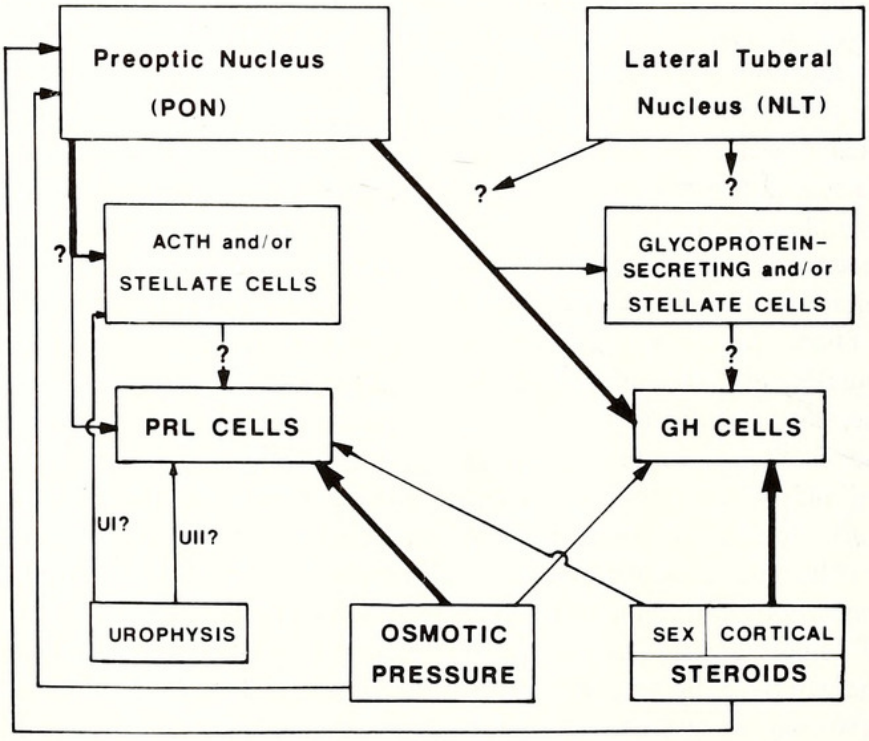

FIG. 1. Pathways for the control of PRL and GH secretion in tilapia. Thick lines (arrows) denote important pathways of control and thin lines indicate pathways of less importance. Question marks denote possible "paracrine" influences and less certain pathways. UI is a CRF-like peptide with possible influence on ACTH cells. UII shares an important tripeptide sequence with somatostatin and inhibits PRL but not GH secretion.

tion in tilapia parallels that for PRL. Unlike the indirect innervation seen in PRL cells, however, ultrastructural observations of the proximal pars distalis (PPD) indicate direct aminergic and peptidergic innervation of the GH cells by hypothalamic fibers [9] (Fig. 1). Fiber types A and B (and C?) are in close proximity to GH cells and often show synaptoid contacts; in addition, some appear to be in direct apposition to the cells of the PPD and PI [21].

Patterns of innervation similar to tilapia's, but with expected species differences, have been described for the goldfish (*C. auratus*) [18, 19], the killifish (*F. heteroclitus*) [17], the medaka (*Oryzias latipes*) [22], the molly (*Poecilia latipinna*) [20, 23], the longjawed mudsucker (*Gillichthys mirabilis*) [15], and the mullet (*Mugil cephalus*) [24].

In less advanced teleosts, such as eels and salmonids, there are noticeable differences in the innervation of the AH. In the European eel (*Anguilla anguilla*), the AH and NH are separated by a basement membrane as in other teleosts, but the penetration of the NH into the AH is not so extensive as is observed in the tilapia, the molly, and other more advanced teleosts, especially in the region of the PPD [2]. Furthermore, control of the PD is reported to be at least partially exerted via hypothalamic nerve endings on hypothalamic arteries in the rostral NH leading to the PD in the Atlantic salmon, *Salmo salar* [25] and brook trout, *Salvelinus fontinalis* [4].

Thus, although teleosts have in common a similar scheme for communication between the brain and the pituitary secretory cells (via direct innervation), there is much variation in the scheme among members of the vast taxonomic grouping termed "Teleostei".

HYPOTHALAMIC CONTROL

Early studies of pituitary transplantations that suggested control of PRL secretion in teleosts by a hypothalamic PRL release-inhibiting factor (PIF) (see [5, 10, 21, 26–29]), and other investigations that demonstrated a direct hypothalamic innervation of the teleost PD [2, 9, 15, 24, 30], prompted interest in the nature of hypophysiotropic factors operating in the hypothalamo-hypophysial area in teleosts [31]. Indeed, many factors have now been implicated in the control of teleost PD function.

Aminergic factors

The role of catecholamines, especially dopamine (DA), in the control of pituitary function in vertebrate tetrapods is well documented; in fact, DA is considered by many to be the vertebrate PIF (cf. [32]). In teleosts, however, the nature of the innervation of the pituitary by aminergic fibers has been unclear. Initial studies described type B fibers either directly innervating the PRL cells in *G. mirabilis* [15, 33] and in *P. latipinna* [20] or ending on the adjacent membrane of the NH in tilapia [15]. However, when the formaldehyde-induced fluorescence technique for monoamines (a definitive method for the identification of catecholaminergic material [34]) is applied to the pituitary of tilapia [9] and *G. mirabilis* [35], none of the type B fibers initially observed in the NH fluoresces positively. Thus, in tilapia, *G. mirabilis*, and possibly other species, catecholamines appear to be absent from the pituitary. In *C. auratus*, however, using ^{3}H–DA, type B fibers have been demonstrated going to and in close apposition to the basement membrane [36], a result supported by studies using a specific antibody against DA [37]. DA antiserum has also been used on the cyprinodont, *Cynolebias whitei*, to demonstrate DA-ergic fibers in the NH [38]. Furthermore, Halpern-Sebold *et al.* [39] have detected tyrosine hydroxylase-immunoreactivity in the hypothalamus and pituitary of *Xiphophorus maculatus*, particularly in the PRL cells. Serotonin, on the other hand, although not observed in fibers in the pituitary of *P. latipinna* using autoradiographic techniques [40], has not been demonstrated in the pituitary of *C. whitei* using serotonin antiserum [38].

Studies of the actions of aminergic factors on PRL secretion using physiological and pharmacological agents *in vitro* have demonstrated effects of DA, serotonin and adrenalins in various teleost species. An inhibitory effect of DA on PRL release is reported in the tilapia [41, 42], *P. latipinna* [23, 43], *G. mirabilis* [41], *C. auratus* [44], *A. anguilla* [45, 46], and *Salmo gairdneri* [47, 48]. In addition, endogenous levels of catechol-

amine presumably act to inhibit PRL secretion from the pituitary gland autotransplanted into the anterior chamber of the eye of *P. latipinna* [49]. Pharmacological agents that affect dopaminergic systems, as well as DA precursors, have been tested in various teleosts and have the expected actions based on mammalian studies; these agents (ergocryptine, 6-hydroxydopamine, L-dopa, reserpine, and various receptor antagonists) and their actions in teleosts are listed in Table 1. Serotonin, on the other hand, is stimulatory to PRL secretion *in vitro* in *S. gairdneri* [48] and in *A. japonica* [50]; in addition, injection of 5-hydroxytryptophan, a serotonin precursor, appears to activate PRL cells in the same species [48, 51]. Furthermore, the intraperitoneal injection of pargyline, a monoamine oxidase inhibitor, results in elevated brain serotonin concomitant with increased pituitary PRL levels in *C. auratus* [52]. In contrast, parachlorophenylalanine, a tryptophan hydroxylase inhibitor, reduces hypothalamic serotonin content in *A. anguilla* [53] and in *S. gairdneri* [54]. The few studies on the role of adrenalins in the control of teleost PRL secretion are apparently contradictory. In *C. auratus,* epinephrine and norepinephrine increase adenylate cyclase activity in PD homogenates [55]. In *P. latipinna,* however, phenylephrine (α-adrenergic agonist) and isoproterenol (β-adrenergic agonist) inhibit PRL secretion *in vitro*; furthermore, the adrenergic blocking agents phentolamine and propranolol have no direct effect on PRL secretion, but oppose DA inhibition of PRL secretion *in vitro* [43].

Aminergic control of GH secretion in teleosts has received relatively little attention. In contrast to its inhibitory effect on PRL secretion, DA

TABLE 1. Factors affecting the dopaminergic control of prolactin secretion

Species	Dopamine	L-Dopa	Ergo-cryptine	Reserpine	6-HODA	Receptor antagonist
Anguilla anguilla						
45*		−	−		+	
140				?		
141		−				
142			−			
Gillichthys mirabilis						
41		−		+	+	
143				+		
Heteropneustes fossilis						
144				+		
Mugil platanus						
145				+	+	
Poecilia latipinna						
43	−					+
146		−			+	+
147			−			
Salmo gairdneri						
47	−					
48	−	−				+
Oreochromis mossambicus						
41	−					
42	−					
148					+	
Xiphophorus helleri						
147			−			

* Numbers under species names are references.

appears to be stimulatory to GH secretion in *C. auratus*; by using various agents administered intraperitoneally and intraventricularly, Chang *et al.* [56] have shown that DA may act centrally to stimulate GH secretion. Wigham *et al.* [43], however, report an *in vitro* inhibition of secretion of the putative GH in *P. latipinna* by DA, although this inhibition is not opposed by the specific DA antagonist 3, 4-dimethylphenylethylamine. Norepinephrine, on the other hand, may act directly to inhibit GH secretion in *C. auratus* [56]. In *P. latipinna,* however, the adrenergic blocker propranolol inhibits GH secretion, whereas phentolamine and other adrenergic pharmacological agents have no *in vitro* effect [43].

Peptidergic agents

The demonstration of a lack of aminergic fluorescence in the hypophysial area of tilapia and *G. mirabilis* raises questions about the nature of the PIF (and/or other factors) in these and other teleosts (see [57]). More recently, a role for somatostatin (SRIF) as an inhibitor of PRL secretion in teleosts has been investigated. By immunocytochemical techniques, SRIF-like material is observed in the brain of the catfish (*Heteropneustes fossilis*) [58] and *S. gairdneri* [59], and can be further localized to the hypothalamus and NH near the PD in *F. heteroclitus, G. mirabilis* [60], tilapia [60, 61], *P. latipinna* [62, 63], and in several other freshwater and seawater teleosts [64–67]. In addition, Batten [63] suggests that the SRIF-immunoreactive (IR) fibers in the pituitary of *P. latipinna* appear to correspond to a particular class of type A fibers: "type A2" identified by electron microscopy (EM) [20]. A similar situation exists in tilapia, where the SRIF-IR fibers in the NH appear to correspond well with the distribution of type C fibers as detected by EM.

In addition, immunocytochemical studies of the hypothalamo-hypophysial system following acute changes in environmental salinity offer further evidence for the role of SRIF in control of PRL secretion in tilapia [68]. Preliminary observations indicate that shortterm (up to 3 hr) transfer from SW to FW results in increased SRIF-IR in the cell bodies of the PON and decreased SRIF-IR in the neurohypophysial processes penetrating the RPD. These observations suggest that transport of SRIF from the PON is inhibited in FW. The reciprocal transfer (FW to SW), on the other hand, is seen to deplete SRIF-IR in the cell bodies of the PON and increase SRIF-IR in the neurohypophysial fibers; furthermore, in SW-transferred tilapia, SRIF-IR is more prominent in the NH, and fibers containing SRIF-IR appear to penetrate the RPD more deeply than in FW-transferred tilapia. SRIF may be the significant PIF in tilapia based on its potent activity *in vitro*.

In tilapia, SRIF is inhibitory to PRL secretion *in vitro* [42, 69, 70], and this inhibitory effect is at least partially independent of any effects of SRIF on PRL synthesis [71]. In *P. latipinna,* SRIF inhibits total and newly-synthesized PRL secretion *in vitro* [72] and reduces synthetic activity of the PRL cells as detected by EM [73]. Coupled with observations on salmonids and eels that indicate less penetration of SRIF-IR hypothalamic fibers into the pituitary [66] as compared with more advanced teleosts, it is interesting that SRIF does not inhibit PRL secretion *in vitro* in *S. gairdneri* [48].

GH secretion is similarly inhibited by SRIF *in vitro* in *Anguilla japonica* [50]. In tilapia, SRIF inhibits GH secretion *in vitro* [70, 74], even in the presence of cortisol which stimulates GH secretion [75]. Helms *et al.* [76] have shown that SRIF is a more effective inhibitor of GH secretion in smaller (*ca.* 60 g) than in larger (*ca.* 120–180 g) tilapia. Using a perifusion system, Marchant *et al.* [77] have shown that SRIF (and SRIF–28) inhibit GH secretion in *C. auratus*; in the same study, however, it was found that catfish pancreatic SRIF–22 has no effect on GH secretion. Furthermore, injection of SRIF lowers plasma GH levels in *C. auratus* [78] as well as in *Oncorhynchus kisutch* [79].

A role for thyrotropin-releasing hormone (TRH) in the hypothalamic control of teleost PRL and GH secretion is supported by recent evidence. In *S. salar sebago,* TRH-IR is observed in the brain and pituitary [80]. TRH stimulates PRL secretion *in vitro* in *A. japonica* [50]. In *P. latipinna,* TRH stimulates PRL secretion *in vitro,* even in a hyperosmotic medium [72], and evidence of increased synthetic activity is observed by EM

[73]. Similarly, Prunet and Gonnet [81] have shown that TRH stimulates PRL secretion *in vitro* in a dose-dependent manner in *S. gairdneri*. Barry and Grau [82] have observed a stimulation of PRL secretion *in vitro* by TRH from pituitaries pretreated with 17β-estradiol. James and Wigham [48] have not observed an *in vitro* effect of TRH on PRL secretion in *S. gairdneri*; however, P. Prunet and F. Gonnet (personal communication) have shown that the addition of a protease inhibitor (e.g., Bacitracin) to the incubation medium prevents breakdown of TRH, allowing TRH to stimulate PRL secretion in a dose-related fashion in the same species.

The *in vitro* effects of vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI) have recently been examined in tilapia [83]. VIP and PHI are potent stimulators of PRL secretion in tetrapods *in vivo* and *in vitro* (cf. [84]), including in an amphibian [85]. In tilapia, however, the first non-tetrapod species in which VIP and PHI have been tested, both VIP and PHI inhibit PRL secretion *in vitro*; these two peptides have no effect, however, on GH secretion. Preliminary immunocytochemical observations suggest a moderate amount of VIP-IR, but no definitive PHI-IR, in the hypothalamo-hypophysial area of tilapia (R. S. Nishioka *et al.*, unpublished; see [83]). Such a discordant pattern of secretion between fish and mammal is not readily explainable, and studies are being conducted presently to gain some understanding of this phenomenon.

In tilapia, other peptide factors have been investigated for their *in vitro* effect on PRL and/or GH secretion. Urotensin II (UII), a dodecapeptide showing sequence similarity to SRIF (sharing an important tripeptide, Phe-Trp-Lys), inhibits PRL secretion, but does not have any significant effect on GH secretion [69, 70]. Recently, Kewish *et al.* (personal communication) have determined that growth hormone-releasing hormone (GHRH) stimulates GH release.

Peptidergic factors of potential importance in the control of teleost PRL and GH secretion are suggested by some immunocytochemical and other studies. Studies on gonadotropin-releasing hormone (GnRH; LHRH) in teleosts, for example, suggest a potentially important role for GnRH. In *S. gairdneri*, immunoreactive GnRH is observed within telencephalic perikarya and in fibers passing to the pituitary stalk [86], whereas in *P. latipinna*, GnRH-IR fibers are observed in the NH leading toward and contacting the gonadotropes in the PPD [63] where GH cells are nearby. Similarly, Kah *et al.* [87] have detected GnRH-IR fibers going to the PPD of *C. auratus*. These anatomical studies of GnRH have prompted our interest in the potential effect of GnRH on tilapia PRL and GH secretion. Furthermore, inasmuch as gonadotropin-associated peptide (GAP), a segment of the GnRH precursor, belongs to the same family of peptides as VIP and PHI [84], we have begun investigation of the effects of this peptide (and some of its cleavage products) using our *in vitro* system (J. Planas *et al.*, unpublished). Corticotropin-releasing factor (CRF), as well, is observed in areas suggestive of a possible relation to PRL or GH secretion. In *C. auratus* and *Cyprinus carpio*, CRF-IR perikarya are observed in the PON and PVN, with fibers from them leading to the pituitary and ending anterior to the ACTH cells of the RPD [67]; the possibility of the intermingled PRL cells of the RPD being affected by such an innervation is suggested. Similar distributions of CRF-IR have been observed in *S. gairdneri* [88, 89] and *P. latipinna* [63]. Combined with the fact that some teleosts are known to secrete PRL while under stress (P. Prunet and M. Avella, personal communication), these CRF immunocytochemical data make further *in vitro* work desirable. Furthermore, urotensin I, a CRF-like peptide from the fish caudal neurosecretory system, and possibly also associated with the family of substances that includes VIP and PHI [84], is another conceivably important peptide factor, as there are claims for its presence in the brain in *Catostomus commersoni* [90]. Finally, in *P. latipinna*, fibers immunoreactive for the two neurohypophysial octapeptides, arginine vasotocin and isotocin, originate from separate preoptic perikarya and end near all AH cell types (except PRL cells) [63]; the possible interaction between neurohypophysial octapeptides which stimulate ACTH secretion *in vitro* in *C. auratus* [91], and the secretion of PRL and GH, is unknown in teleosts. Thus, several peptide factors of potential importance in the control of

teleost PRL and GH secretion merit further study.

EXTRAHYPOTHALAMIC CONTROL

In addition to hypothalamic factors, various extrahypothalamic factors also appear to control PRL and GH secretion in teleosts (see [3, 92–94]). These factors, which include medium osmotic pressure, sex steroids, corticosteroids and thyroid hormones, also appear to modulate the control of PRL and GH secretion.

Osmotic factors

In a pioneering study, Pickford and Phillips [95] demonstrated the important role of PRL in FW osmoregulation; subsequent investigations on numerous species of teleosts have substantiated the importance of PRL in FW adaptation (see [28, 92, 96–98]). There is general consensus that low osmotic pressure, characteristic of FW, is an important factor stimulating PRL secretion in euryhaline teleosts. Osmotic pressure and PRL secretion *in vivo* and *in vitro* are inversely related (Table 2).

The salmonids present an exceptional group regarding the control of PRL secretion by osmotic pressure. In some salmonid species, it is difficult to demonstrate a direct relationship between osmotic pressure and PRL cell activity *in vitro*, but, in other salmonid species, the typical inverse relationship exists between osmotic pressure and PRL cell activity (Table 3). It has been suggested that this disparity may be due to the differences of the stage of development of the individuals used in a particular study [99]. Upon scrutiny of the studies listed in Table 3, there appears to be a tendency for decreased ability to regulate PRL secretion with advances in development; PRL cells of alevins, parr and smolts are responsive, whereas those of mature fish of some species are less responsive.

In addition, there appear to be differences in responses *in vivo* and *in vitro* . For example, PRL secretion *in vitro* from the PD of adult *O. keta* is unresponsive to variations in medium osmotic pressure [100], whereas plasma levels of PRL respond to changes in osmotic pressure *in vivo* [101]. These studies suggest that some factor in intact *O. keta* (possibly hypothalamic) is absent *in vitro*. On the other hand, Cook and van Over-

TABLE 2. Fishes showing inverse relationship between PRL cell activity and medium osmotic pressure and/or ion concentration

Species	Reference
Anguilla anguilla	105, 107, 108, 109, 149, 150
A. japonica	50, 111
Aphanius dispar	151, 152
Carassius auratus	18, 111, 153
Cichlasoma biocellatum	154
Fundulus heteroclitus	95, 155, 156
Gasterosteus aculeatus	157, 158
Gillichthys mirabilis	21, 41, 159
Lebistes sp.	160
Mugil sp.	30, 161
Oreochromis mossambicus	49, 97, 119, 126, 148, 162, 163, 164
Oryzias latipes	111, 165, 166
Platichthys stellatus	159
Poecilia sp.	20, 49, 107, 150, 168, 169, 170
Tilapia sp.	118
Xiphophorus sp.	171, 172, 173, 174, 175, 176, 177, 178, 179

TABLE 3. Prolactin cell activity at low osmotic pressure in salmonids based on various criteria

Species	Age	Response	Criterion	Reference
Oncorhynchus keta	adult	+	RIA (plasma)	101
(chum salmon)	adult	−	incubation	100
O. kisutch	alevin	+	cytology	180
(coho salmon)	fry	+	cytology	181
	smolt	+	cytology	99
	"yearling"	+	RIA (plasma & RPD)	182, 183
	parr/smolt	+	incubation	182
Oncorhynchus nerka	smolt	−	cytology	114
(sockeye salmon)	smolt	+	RIA (plasma)	103
	adult	−	cytology	102
Salmo gairdneri	adult	−	incubation	108
(rainbow trout)	"yearling"	+	RIA (plasma)	184
	"yearling"	− (?)	incubation	185

beeke [102] and McKeown and Leatherland [103] found no *in vivo* responsiveness in adult *O. nerka* subjected to different osmotic environments.

Thus, although it may be generally true that euryhaline teleosts can respond to osmotic pressure changes by altering PRL secretion, there are some exceptions to this rule among salmonids. Further study of this group is needed, with special attention to various life stages.

The catadromous *A. anguilla* [104–110] and *A. japonica* [100, 111] respond to lower osmotic pressure *in vivo* and *in vitro* by increasing PRL secretion at any stage of development. The anadromous *Gasterosteus aculeatus* has reduced PRL cell activity *in vivo* when exposed to FW containing Ca^{++} and Mg^{++} equal to that of SW [112]. Seasonal differences in PRL cell secretory cycle have been reported by Lam and Leatherland [113].

GH secretion, on the other hand, has received less attention regarding the influence of osmotic pressure. In two salmonid species, *O. nerka* [114] and *O. keta* [115], and in *P. latipinna* [116], there are no detectable changes in GH secretion *in vitro* in response to osmotic pressure changes of the medium. In *S. gairdneri,* however, large shortterm increases in the sodium content of the ambient medium inhibit GH secretion [108]. Similarly, in *A. anguilla,* high sodium medium inhibits and low sodium medium stimulates GH secretion *in vitro* [108]. These results are supported by cytological studies of the GH cells in *A. anguilla* [104, 110, 117] and *A. japonica* [100, 111]. Furthermore, in two other tilapia species, *Tilapia grahami* and *T. alcalica,* the GH cells appear more active in fish acclimated to FW than in fish from African "soda" lakes [118]. However, Zambrano *et al.* [119] reported no ultrastructural changes in the GH cells of 20–30 g tilapia after transfer from SW to FW. In contrast, Helms *et al.* [76] have observed increased GH secretion *in vitro* in response to increased osmotic pressure in tilapia weighing *ca.* 60 g, but not in larger fish (*ca.* 120 g). The fact that GH has been shown to promote hypoosmoregulatory ability in salmonids (see [120] for references) suggests a possible role for GH in SW osmoregulation in these fish. At present, no generalization on the control of GH secretion by osmotic pressure is possible.

Hormones

Extrahypothalamic factors other than osmotic pressure have also been implicated in the control of PRL and GH secretion. Prolactin itself, by injection or as a result of uninhibited release from transplanted pituitaries, may have an inhibitory effect on *in situ* PRL cells (see [28]). Cortisol, which is believed to be a SW-adapting hormone in some teleosts, inhibits PRL secretion *in vitro* [49] and stimulates GH secretion *in vitro* [75, 76] in tilapia (Fig. 1). In contrast, cortisol is without effect on *in vitro* PRL secretion in *S. gairdneri* [48].

D, L-thyroxine inhibits GH release *in vitro* in *A. anguilla* [108], although triiodothyronine does not have any effects on *in vitro* GH secretion in tilapia ([75]; B. Kewish *et al.*, personal communication). 17β-Estradiol stimulates PRL synthesis [49] and promotes stimulation of secretion of PRL by TRH in tilapia [82]. Estradiol also stimulates PRL secretion in *A. japonica* [100]. In *C. auratus,* treatment of females *in vivo* with synthetic estrogen (ethinylestradiol) [111] or with 17β-estradiol [121] causes an increase in GH cell activity as detected by EM. Similarly, Young and Ball [122] found GH cells in *P. latipinna* strongly activated by 17β-estradiol treatment. B. Kewish *et al.* (personal communication) have recently tested methyltestosterone in male tilapia, and found no effect of this steroid *in vitro*. γ-Amino-n-butyric acid has also been tested in tilapia by Wigham *et al.* [49], and no effect on PRL secretion *in vitro* was observed.

Intracellular mediators

Although increased PRL secretion was once ascribed to a direct effect of low environmental Ca^{++} rather than to low osmotic pressure and/or Na^{+} concentration in *G. aculeatus* by Wendelaar Bonga [112] and in tilapia by Wendelaar Bonga and van der Meij [123, 124], these authors subsequently proposed that their earlier conclusions of stimulation of PRL secretion by low cation levels *in vivo* may have resulted from an indirect effect of Ca^{++} concentration on gill permeability [125]. Furthermore, other workers have observed that PRL secretion *in vitro* is independent of physiological Ca^{++} concentration in tilapia [69, 97, 126], in *P. latipinna* [116], in *O. kisutch* [127] and in *S. gairdneri* (L. R. Johnston and T. Wigham, personal communication), provided a minimal level is present. MacDonald and McKeown [127] have found an optimal concentration of Ca^{++} for promoting PRL secretion *in vitro* in *O. kisutch,* and this concentration is roughly equal to the physiological levels of Ca^{++} found in the plasma of *O. nerka* [128].

Evidence for a role of Ca^{++} as an intracellular mediator of teleost PRL secretion is provided by various *in vitro* studies. For example, Taraskevich and Douglas [129] report that spontaneous secretion of PRL from the RPD of the teleost *Alosa pseudoharengus* is associated with action potentials partly mediated by extracellular Ca^{++}. Similarly, exposure of tilapia PRL cells to a depolarizing concentration of K^{+} elevates PRL secretion, presumably through the opening of Ca^{++} channels [130]. Further work on tilapia is in accord with these data: exposure of PRL cells to the Ca^{++} ionophore A23187 elicits increased PRL secretion, even in the presence of SRIF [69], and exposure of PRL cells to D600 (an organic Ca^{++} channel blocker), blocks K^{+}-induced PRL elevation [130]. Interestingly, D600 does not have much effect on hypoosmotic medium-induced PRL secretion, suggesting that the effects of osmotic pressure on PRL release may be mediated by mechanisms different from those operating during chronic depolarization [130].

The mode of action of Ca^{++} in intracellular mechanisms is unclear, although a few studies suggest some particular roles for Ca^{++}. An action of Ca^{++} distal to its influx through a voltage-regulated channel may occur, as the use of Co^{++}, a competitive inhibitor of Ca^{++} in various calcium-mediated processes [131], suppresses PRL secretion induced by hypoosmotic medium in tilapia [130], as well as by K^{+}-depolarizing medium (N. H. Richman and E. G. Grau, personal communication). On the other hand, *in vitro* exposure of the pituitary to chlorpromazine, a drug that may act on a Ca^{++} gate involving phosphatidyl inositol turnover [132–134] and that stimulates PRL secretion in mammals [135], simultaneously stimulates ^{3}H-PRL secretion and decreases $^{45}Ca^{++}$ accumulation in the coho salmon pituitary [136].

Post-receptor mechanisms utilizing the second messenger cAMP have been studied in teleost PRL cells by various groups. In tilapia, PRL secretion *in vitro* is stimulated by treatment with dibutyryl cyclic AMP (db-cAMP) alone, with 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor) alone, or with a combination of the two [69]. In *S. gairdneri,* db-cAMP stimulates both synthesis and release of PRL *in vitro* [47] and, in *O. kisutch,* db-cAMP stimulates synthesis, but not secretion, of PRL *in vitro* [136]. Similarly, L. M. H. Helms *et al.* (personal com-

munication) have stimulated PRL release with forskolin (adenylate cyclase stimulator) in tilapia, and L. R. Johnston and T. Wigham (personal communication) have demonstrated in *S. gairdneri* a db-cAMP stimulation of PRL synthesis, but not release, as well as a forskolin stimulation of PRL secretion and cAMP production. Recently, in preliminary studies on the striped bass (*Morone saxatilis*) in our laboratory, 8-bromo-cAMP, IBMX, and forskolin have proven to be equally stimulatory on PRL secretion *in vitro* (R. S. Nishioka *et al.*, unpublished).

A relationship between Ca^{++} and the adenylate cyclase-cAMP system, thought to possibly operate via Ca^{++}/calmodulin-induced cAMP formation [137–139], has not been studied directly in teleosts. However, MacDonald and McKeown [136] report a net $^{45}Ca^{++}$ accumulation in *O. kisutch* RPD tissue treated with db-cAMP, and Grau *et al.* [69] can reverse SRIF inhibition of PRL secretion by treatment with A23187, a calcium ionophore; thus, these studies suggest a relationship between Ca^{++} and cAMP, possibly via calmodulin, meriting further investigation.

PERSPECTIVES

As stated earlier, neuronal processes from the hypothalamus do not directly contact the PRL cells in tilapia. Generally, a layer of ACTH and stellate cell processes is interposed. It is possible that both these cell types play a "paracrine" role in stimulus transmission (Fig. 1). Since stellate cells with slender processes are closely apposed to PRL cells throughout the RPD of freshwater tilapia, their paracrine involvement could be substantial. Indeed, stellate cells with prominent processes stand out among the condensed PRL cells (under maximal inhibition) in seawater-adapted tilapia. Stellate cells and gonadotropic/thyrotropic cells could play a similar role in regard to GH secretion (Fig. 1).

The diversity of factors controlling PRL and GH secretion and release in tilapia and other teleosts is apparent in this review. It is possible that some of these molecules may have coincidental structural similarities in minor amino acid sequences and/or spatial configuration that may "fit" a particular receptor. If this be true, then a substance foreign to the organism may cause a response in a fashion indistinguishable from that of a bonafide agent. Currently, many substances have been found to have inhibitory or stimulatory activity, but it is unlikely that all of these compounds originate from the hypothalamus of a teleost. On the other hand, redundancy of control may be built into these systems to allow separate maintenance of osmoregulatory, reproductive, and other physiological pathways.

The continued utilization of isolated RPD and PPD of teleosts in general for *in vitro* studies offers many advantages. PRL cells in the RPD and GH cells in the PPD are segregated into nearly homogeneous masses which are easy to dissect and convenient for use in incubation (or perifusion). These cell masses can be dissociated to provide uniform cell populations for a variety of studies. In addition, the secretory activity of PRL cells and some GH cells can be manipulated simply by altering the tonicity of the medium, thus facilitating the analysis of inhibitory and stimulatory control.

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