Endocrine Control of Osmoregulation in Teleost Fish[1]

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SYNOPSIS.   As the primary link between environmental change and physiological response, the neuroendocrine system is a critical part of osmoregulatory adaptations. Cortisol has been viewed as 'the' seawater-adapting hormone in fish and prolactin as 'the' fresh water adapting hormone. Recent evidence indicates that the growth hormone/insulin-like growth factor I axis is also important in seawater adaptation in several teleosts of widely differing evolutionary lineages. In salmonids, growth hormone acts in synergy with cortisol to increase seawater tolerance, at least partly through the upregulation of gill cortisol receptors. Cortisol under some conditions may promote ion uptake and interacts with prolactin during acclimation to fresh water. The osmoregulatory actions of growth hormone and prolactin are antagonistic. In some species, thyroid hormones support the action of growth hormone and cortisol in promoting seawater acclimation. Although a broad generalization that holds for all teleosts is unlikely, our current understanding indicates that growth hormone promotes acclimation to seawater, prolactin promotes acclimation to fresh water, and cortisol interacts with both of these hormones thus having a dual osmoregulatory function.

The capacity to regulate plasma ions in the face of changing external salinity is an obvious necessity for fish that live in estuaries or that move between fresh water and seawater as part of their normal life cycle. The need to respond to salinity change may be rapid, such as during tidal cycles or rapid movements through estuaries, or slow, such as in the seasonal or ontogenetic acquisition of salinity tolerance in anadromous fish. The former requires the rapid activation of existing mechanisms (transport proteins and epithelia), whereas the second requires the differentiation of transport epithelia and synthesis of new transport proteins. As the primary link between environmental change and physiological response, the neuroendocrine system is a critical part of these osmoregulatory adaptations. In this paper I will review recent evidence for the hormones involved in development and differentiation of transport epithelia that control the ability of teleost fish to move between fresh water and seawater. Our previous "textbook view" of the endocrinology of osmoregulation has been that cortisol is "the" seawater-adapting hormone and prolactin is "the" fresh water-adapting hormone, clearly defined for the first time by Utida *et al.* (1972). Evidence collected in the last 15 yr indicates that the growth hormone/insulin-like growth factor I (GH/IGF-I) axis is also important in the seawater acclimation process of teleosts. Recent findings on the importance of cortisol in ion uptake will also be presented, and these indicate that cortisol has a dual osmoregulatory function in many teleosts.

THE PHYSIOLOGY OF EURYHALINITY

Irrespective of the salinity of their external environments, teleost fishes maintain their plasma osmotic concentration about one-third that of seawater. In fresh water this requires counteracting the passive gain of water and loss of ions by producing a copious dilute urine and actively taking up ions across the gills. In seawater, teleosts must counteract the passive gain of ions and loss of water. This is accomplished by drinking seawater, absorbing water and salts across the gut, and secreting excess monovalent ions across the gills and divalent ions

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through the kidney. Evans (1984) has estimated that 95% of teleost species are stenohaline, living wholly in either fresh water or seawater. The remaining 5% are euryhaline, having the capacity to withstand large changes in environmental salinity, a trait that is widespread among teleost lineages and has apparently evolved many times. This capacity to evolve euryhalinity may be one reason that teleosts can be found in almost all aquatic habitats.

The mechanisms of ion transport in teleost fish have been the subject of several recent reviews (Evans *et al.*, 1999; Marshall and Bryson, 1998). As outlined above, the gills are the primary site of net sodium and chloride transport, actively taking up salts in fresh water and secreting them in seawater. Most of the recent work on the endocrine control of ion transport in fish has focused on the gill, so this review will necessarily be biased in this direction (see Utida *et al.*, [1972] for a review of the endocrine control of the gut and urinary bladder). It has been known for some time that the mitochondrion-rich chloride cell is the site of salt secretion (Foskett and Scheffey, 1982*b*). There is substantial evidence indicating that the major transporters involved in salt secretion in the gill includes basolaterally located Na^+, K^+-ATPase (the sodium pump) and Na^+, K^+, $2Cl^-$ cotransporter (NKCC), and an apical Cl^- channel that appears to be homologous with the cystic fibrosis transmembrane conductance regulator (see Fig. 1). The site and mechanims involved in ion uptake in fresh water are less certain. Both chloride cells and pavement cells may be involved in sodium and chloride uptake. Chloride is exchanged for HCO_3^- at the apical surface and leaves at the basolateral membrane moving 'downhill' on an electrical gradient (the chloride cell being more negative than the blood). Sodium may enter the gill epithelia by exchange with H^+, or through an apical Na^+ channel coupled to an apical H^+-ATPase, and then leave at the basolateral surface through Na^+, K^+ATPase. Recent evidence suggests that H^+-ATPase and the apical sodium channel are located on the apical surface of pavement cells in Mozambique tilapia (*Oreochromis mossambicus*), but in

both pavement cells and chloride cells in the rainbow trout (*Oncorhynchus mykiss*) (Hiroi *et al.*, 1998; Wilson *et al.*, 2000). Most of our knowledge of the transporters involved in ion uptake and secretion in fish comes from ion substitution and phamacological studies. With the exception of the sodium pump and the apical chloride channel, the sequence and physical structure of transporters involved in ion transport in fish have not been characterized (see Evans *et al.*, 1999). More direct evidence is needed to establish the roles and location of these transporters in teleosts.

THE ROLE OF THE GH/IGF-I AXIS IN ACCLIMATION TO SEAWATER

Evidence for the importance of the GH/IGF-I axis in seawater acclimation comes primarily from studies on exogenous hormone treatment, changes in circulating levels, metabolic clearance rate, localization of receptors and production of IGF-I by osmoregulatory tissues (Sakamoto *et al.*, 1993; Mancera and McCormick, 1998*a*). Komourdjian *et al.* (1976) found that long term GH treatment increased both the size and salinity tolerance of Atlantic salmon (*Salmo salar*). Recent studies of growth hormone transgenic salmon also show that these larger fish have increased salinity tolerance (Saunders *et al.*, 1998; Devlin *et al.*, 2000). Because larger salmonids have inherently greater salinity tolerance, it was not clear whether these effects of GH were specific to osmoregulation or were indirect through the growth effects of GH. Bolton *et al.* (1987) found that a single injection of GH in rainbow trout (*Oncorhynchus mykiss*) followed two days later by exposure to seawater resulted in increased salinity tolerance. This time course was too rapid to be explained by changes in body size, thus indicating that GH has osmoregulatory actions independent of its effect on growth. Subsequent research found that GH could increase salinity tolerance in many salmonid species (Sakamoto *et al.*, 1993). At least some of the osmoregulatory actions of GH are carried out by IGF-I. Using a protocol similar to that of Bolton *et al.* (1987), McCormick *et al.* (1991*b*) found that IGF-I increased salinity tolerance in rainbow trout,
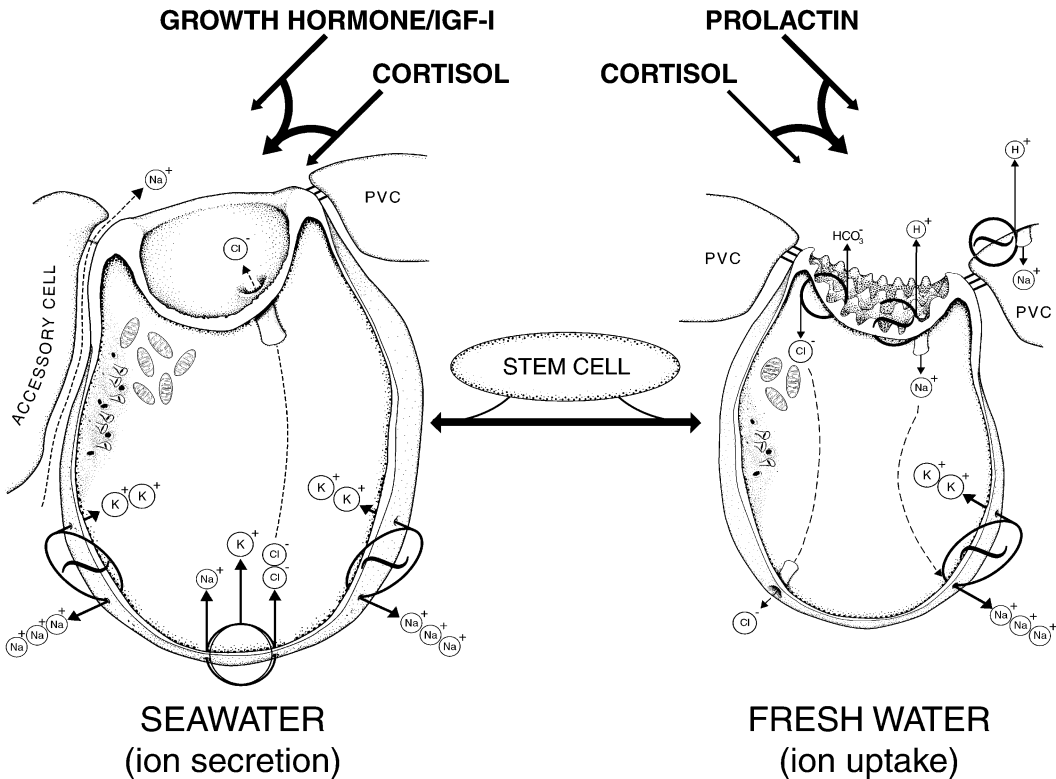
FIG. 1. Morphology and transport mechanisms of gill chloride cells in seawater and fresh water. See text for details of transport mechanisms. Chloride cells are characterized by numerous mitochondria and an extensive tubular system that is continuous with the basolateral membrane. In seawater, chloride cells are generally larger and contain a deep apical cyrpt, whereas in freshwater the apical surface is broad and contains numerous microvilli. In some species, such as tilapia the H^+-ATPase and apical sodium channel may be present in pavement cells rather than chloride cells. Recent evidence suggests that individual chloride cells can move between these two mophological states (Hiroi *et al.*, 1999), and also arise from undifferentiated stem cells (Wong and Chan, 1999). Growth hormone and cortisol can individually promote the differentiation of the seawater chloride cell, and also interact positively to control epithelial transport capacity. Prolactin inhibits the formation of seawater chloride cells and promotes the development of fresh water chloride cells. Cortisol also promotes acclimation to fresh water by maintaining ion transporters and chloride cells, and by interacting to some degree with prolactin. PVC = pavement cell.

and this effect was later confirmed in other salmonids (McCormick, 1996; Seidelin *et al.*, 1999).

For some time it was thought that the osmoregulatory actions of GH and IGF-I were related to the spring migration and growth cycle of anadromous salmon and perhaps restricted to salmonids. Recent evidence, however, indicates that GH/IGF-I effects on salinity acclimation may be widespread among teleosts. GH injection increases hypo-osmoregulatory ability in two cichlid species, the Nile and Mozambique tilapia (*Oreochromis niloticus* and *O.*

mossambicus) (Xu *et al.*, 1997; Sakamoto *et al.*, 1997). Mancera and McCormick (1998*b*) found that both GH and IGF-I injections increased salinity tolerance in the intertidal mummichug, *Fundulus heteroclitus* (Family Cyprinodontidae). Although these represent a small number of the euryhaline species, they are widely separated in the evolution of teleosts, suggesting that the osmoregulatory action of GH and IGF-I may also be widespread among teleosts.

Examination of the mechanisms of action of GH and IGF-I to promote salinity tolerance indicates that the gill is an important

target tissue. GH and IGF-I stimulate the number and/or size of gill chloride cells in salmonids and tilapia (Sakamoto *et al.*, 1993; Xu *et al.*, 1997). Prunet *et al.* (1994) found that GH increased the number of α-chloride cells and accessory cells (both thought to be involved in salt secretion), and decreased the number of β-cells (putative ion uptake cells) in juvenile Atlantic salmon. GH and IGF-I increase gill Na^+, $K^+ATPase$ activity and/or mRNA levels in salmonids, tilapia and mummichog (Madsen *et al.*, 1995; Mancera and McCormick, 1998*b*; Xu *et al.*, 1997; Sakamoto *et al.*, 1997), and immunocytochemical studies indicate that hormone induced increases in Na^+, $K^+ATPase$ are localized to chloride cells (Seidelin *et al.*, 1999). It has recently been shown that GH also upregulates the $Na^+:K^+:2Cl^-$ cotransporter in gill chloride cells of Atlantic salmon (Pelis and McCormick, 2001). To date, regulation of other gill transporters has not been examined.

Although the number of studies are limited, there is no evidence that GH can directly (*in vitro*) increase gill Na^+, $K^+ATPase$ activity (McCormick and Bern, 1989). The ability of IGF-I to increase gill Na^+, $K^+ATPase$ activity and the ability of GH to regulate *in vitro* responsiveness of gill tissue to IGF-I further suggests an indirect action of GH on gill tissue, and a direct action of IGF-I (Madsen and Bern, 1993). Levels of IGF-I mRNA in gill and kidney increases following GH injection and exposure to seawater, indicating that local production of IGF-I may act in a paracrine fashion to influence transport capacity of gill and renal epithelia (Sakamoto and Hirano, 1993). Whether IGF-I acts primarily in an endocrine or paracrine fashion is unknown. *In vivo* studies indicate that IGF-I by itself does not carry out all of the osmoregulatory actions of GH, and that other endocrine factors and/or binding proteins may also be involved (McCormick, 1996).

To date, investigations on the mechanisms of action of the GH/IGF-I axis have almost exclusively focused on the gill, though it is likely that the gut and kidney are also responsive. Fuentes and Eddy (1997) found that GH treatment increased drinking rate after exposure of Atlantic salmon to seawater, suggesting that GH may affect gut function. In brown trout, IGF-I treatment increased gill but not intestinal Na^+, $K^+ATPase$, whereas cortisol increased both (Seidelin *et al.*, 1999).

In addition to the effects of exogenous hormone treatments, changes in the endocrine response to SW also provides evidence for the osmoregulatory actions of GH and IGF-I. This evidence comes primarily from studies on salmonids where the gene sequences of these hormones are known and assays for quantitation of gene expression, circulating levels and receptors have been developed. Plasma levels of GH increase following seawater exposure of coho, chum and Atlantic salmon and rainbow trout (Sakamoto *et al.*, 1993). Metabolic clearance rate of GH in trout is also increased following seawater acclimation (Sakamoto *et al.*, 1990). In Atlantic salmon, plasma IGF-I remains elevated for 2–14 days following seawater exposure (S. D. McCormick, T. Bj. Bjornsson, and S. Moriyama, unpublished results). Hepatic, branchial and renal levels of IGF-I mRNA increase during smolting of coho salmon and following seawater transfer (Sakamoto *et al.*, 1995). In Mozambique tilapia, high concentrations of IGF-I have been found specifically in chloride cells and in epithelial cells of the proximal tubule (Reinecke *et al.*, 1997). GH receptors have been found in the gill and kidney (but not intestine) of coho salmon (Fryer and Bern, 1979; Gray *et al.*, 1990), and this GH binding was decreased in "stunted" juveniles that experience seawater-induced growth retardation. Sakamoto and Hirano (1991) found that the occupancy of hepatic, but not branchial and renal, growth hormone receptors increased following exposure to seawater. A partial sequence and characterization of the IGF-I receptors in fish has been documented (Drakenberg *et al.*, 1993; Elies *et al.*, 1996; Chan *et al.*, 1997; Funkenstein *et al.*, 1997), but there is currently no information on its distribution in transport epithelia.

To date, effects of GH and/or IGF-I on osmoregulation outlined above have been demonstrated in six teleost species distributed among three families (salmonidae, cyprinodontidae and cichladae). Although

these represent a small number of the total number of teleosts, they are widely spread within the teleost lineage: salmonids appeared early in teleost evolution, while *Fundulus* and tilapia belong in the two most recently evolved teleost clades (Atherinomorpha and Percomorpha; Helfman *et al.*, 1997). Since the effects of the GH/IGF-I axis in osmoregulation has only recently been found for these species, it seems likely that more research will establish that these effects are widespread among euryhaline teleosts.

INTERACTION OF THE GH/IGF-I AXIS WITH CORTISOL

The recent evidence for a role of the GH/IGF-I axis does not diminish the importance of cortisol in salt secretion in fish. Several lines of evidence provide substantial support for a role of cortisol in seawater acclimation, including increased circulating levels and metabolic clearance after exposure to seawater, the effects of interrenalectomy, and the effects of cortisol treatment on ion regulation and salinity tolerance in intact, hypophysectomized and interrenalectomized fish (see reviews by Bern and Madsen, 1992; Foskett *et al.*, 1983; McCormick, 1995). Recent evidence indicates that the GH/IGF-I and cortisol axes work together to regulate salt secretion in teleosts. It has been shown for several salmonid species that injection of GH and cortisol together increases gill Na^+, $K^+ATPase$ activity and salinity tolerance to a greater extent than either hormone alone (Madsen, 1990*b*; Madsen and Korsgaard, 1991; McCormick, 1996). This effect can be seen in both hypophysectomized and intact fish (Björnsson *et al.*, 1987; Madsen, 1990*a*). Stimulation of gill Na^+, $K^+ATPase$ activity appears to involve a synergistic action of the two hormones (Madsen, 1990*b*; McCormick, 1996). Similar to the effects reported for salmonids, Mancera and McCormick (1999) found that GH and cortisol together increase hypo-osmoregulatory ability of mummichog to a greater extent than either hormone alone.

It is less clear whether IGF-I and cortisol have a similar capacity to interact. McCormick (1996) found that in Atlantic salmon, IGF-I and cortisol together stimulated gill Na^+, $K^+ATPase$ activity to a greater extent than either hormone alone, but that this interaction was weaker than that for GH and cortisol. In brown trout (*Salmo trutta*), Seidelin *et al.*, (1999) have recently found an additive effect of IGF-I and cortisol on gill Na^+, $K^+ATPase$ activity and mRNA levels, and a significant interaction between the two hormones in increasing the number of Na^+, $K^+ATPase$ immunoreactive cells (chloride cells) in the gill.

One mechanism by which the GH/IGF-I and cortisol axes may interact is through the regulation of cortisol receptors. The cortisol receptor from rainbow trout has recently been cloned (Ducouret *et al.*, 1995), and *in situ* hybridization and immunocytochemical approaches have shown that cortisol receptors are preferentially located in chloride cells and undifferentiated cells (stem cells) in the primary filament of chum salmon (Uchida *et al.*, 1998). GH treatment causes increased numbers of gill cortisol receptors in coho and Atlantic salmon (Shrimpton *et al.*, 1995; Shrimpton and McCormick, 1998). The number of gill cortisol receptors is strongly correlated with the capacity of cortisol to stimulate gill Na^+, $K^+ATPase$ *in vitro* and *in vivo* (McCormick *et al.*, 1991*a*; Shrimpton *et al.*, 1994; Shrimpton and McCormick, 1999), indicating that the regulation of cortisol receptors is physiologically relevant.

Laurent *et al.* (1994) found that growth hormone increased mitotic activity (bromodeoxyuridine labelling) in several cell types in the gill of rainbow trout. Cortisol had no effect or decreased mitotic activity, but increased the number of chloride cells, suggesting that cortisol acts primarily on differentiation of chloride cells. These findings indicate that cortisol and growth hormone may interact, with GH causing general cell proliferation in the gill, creating more stem cells that can then be acted on by cortisol. It would be of interest to examine whether GH increases the number of stem cells with high levels of cortisol receptors as described above.

In addition to the interaction of the GH/IGF-I axis and cortisol at the gill tissue, these endocrine axes may also interact at

''higher'' regulatory pathways, such as the hypothalamus and pituitary. *In vivo* and *in vitro* exposure to GH increases the sensitivity of interrenal tissue to ACTH (adrenocorticotrophic hormone) in coho salmon, causing increased release of cortisol (Young, 1988). Rousseau *et al.* (1999) found that corticotropin releasing hormone is a potent stimulator of *in vitro* growth hormone release in European eel (*Anguilla anguilla*).

Although there is conflicting evidence regarding the role of thyroid hormones in osmoregulation, most studies have found that thyroid hormones by themselves cannot increase ion uptake or secretory capacity (Ayson *et al.*, 1995; Mancera and McCormick, 1999; McCormick, 1995). Exceptions to this are studies in Atlantic salmon, where prolonged thyroxine (T_4) treatment advanced the smolt-related increases in gill Na^+, K^+ATPase activity and the number of chloride cells (Madsen and Korsgaard, 1989), and rainbow trout in which dietary tri-iodothyronine (T_3) increased the number of gill chloride cells without affecting gill Na^+, K^+ATPase activity (Trombetti *et al.*, 1996). Iwata *et al.* (1987) found an inconsistent effect of T_4 on seawater tolerance of chum salmon fry. Peter *et al.* (2000) have recently shown that physiological levels of exogenous T_4 and T_3 in Mozambique tilapia results in increased chloride cell size, gill Na^+, K^+ATPase activity and plasma $[Na^+]$ and $[Cl^-]$, suggesting that thyroid hormones may have a role in ion uptake in this species. Thyroid hormones play at least a supportive role in seawater acclimation, and may interact with both the GH/IGF-I and cortisol axes. Inhibition of the thyroid axis with thiouruea in mummichug caused increased plasma $[Na^+]$ and osmolality in seawater but had no effect in fresh water (Knoeppel *et al.*, 1982). T_4 treatment alone has no effect, but potentiates the action of cortisol on gill Na^+, K^+ATPase activity in tilapia (Dange, 1986), and the action of GH on gill Na^+, K^+ATPase activity in amago salmon (*Oncorhynchus rhodurus*; Miwa and Inui, 1985). Inhibition of the conversion of T_4 to T_3 inhibited normal and GH-induced seawater acclimation in rainbow trout (Lebel and Leloup, 1992; Leloup and

Lebel, 1993). T_3 treatment upregulates the number of gill cortisol receptors in rainbow trout and increases the *in vitro* capacity of cortisol to increase gill Na^+, K^+ATPase activity (Shrimpton and McCormick, 1999). In Atlantic salmon, T_3 increases the number of gill cortisol receptors, and this effect is potentiated when T_3 is administered with growth hormone (Shrimpton and McCormick, 1998). Thyroid hormones thus appear to exert their influence on ion secretory mechanisms primarily through their interaction with the GH/IGF-I and cortisol axes.

THE HORMONAL CONTROL OF ION UPTAKE: A DUAL OSMOREGULATORY ROLE FOR CORTISOL?

There is overwhelming experimental support for the role of prolactin in promoting ion uptake and inhibiting ion secretion in a large and phylogenetically diverse number of teleost species, and this research has been the subject of several reviews (Foskett *et al.*, 1983; Hirano, 1986). Most recent studies support this role of prolactin (*e.g.,* Yada and Ito, 1999), and have also established that prolactin is antagonistic to the actions of GH on salt secretory mechanisms (Madsen and Bern, 1992; Seidelin and Madsen, 1997).

As discussed above, cortisol has largely been identified as a seawater-adapting hormone in a large number of teleost species. There is an increasing body of evidence, however, that cortisol in also involved in ion uptake. Cortisol treatment at physiological doses increases the uptake of sodium in intact and interrenalectomized European eels and intact goldfish in fresh water, affecting both renal and branchial functions (Maetz and Morel, 1965; Mayer *et al.*, 1967; Rankin *et al.*, 1967; Chan *et al.*, 1969). Plasma osmolality of hypophysectomized eels in fresh water is increased after cortisol treatment (Chan *et al.*, 1968). Similarly, plasma ion levels in hypophysectomized goldfish are partially restored by treatment with cortisol (Lahlou and Giordan, 1970). Survival of hypophysectomized *Gambusia* and bowfin (*Amia calva*) in fresh water is increased by treatment with ACTH (Chambolle, 1967; Hanson *et al.*, 1976), which can be presumed to be acting through

its stimulation of cortisol release from the interrenal. ACTH increased plasma sodium levels in intact (but not in hypophysecto-mized) *Fundulus kansae* in fresh water (Stanley and Fleming, 1967). Cortisol is also required to maintain water movement across the gut of fresh water eels (Gaitskell and Chester Jones, 1970). Perry *et al.* (1992) found that cortisol injection of rainbow trout, European eel, Mozambique tilapia and catfish (*Ictalurus nebulosus*) increased the surface area of gill chloride cells and the influx of sodium and chloride. This increased apical surface area is characteristic of putative ion uptake cells, and in these studies was strongly correlated with ion uptake. Cortisol treatment significantly increased the ion regulatory capacity of the gilthead sea bream (*Sparus auratus*) during exposure to low salinity (Mancera *et al.*, 1994). In the medaka (*Oryzias latipes*), cortisol treatment increased the ability of acid-exposed fish to maintain plasma sodium levels (Yada and Ito, 1999).

Changes in circulating levels of cortisol also support a role for this hormone in ion uptake. Transfer of European eel, tilapia, mummichug, carp (*Cyprinus carpio*) and *Chrysophrys major* from seawater to fresh water results in transient increases in plasma cortisol, and transfer of several marine species such as mullet (*Mugil cephalus*), starry flounder (*Platichthys stellatus*), sea bass (*Dicentrachus labrax*) and gilthead sea bream from high to low salinity results in increased circulating cortisol that remains elevated for days to weeks (see references in Mancera *et al.*, 1994). In tilapia, transfer from fresh water to distilled water (Assem and Hanke, 1981), and prolactin injections (Assem and Hanke, 1984) increase the concentration of circulating cortisol. These studies on increasing cortisol levels in response to low salinity along with the cortisol treatment studies described above provide evidence that in many teleosts, cortisol has a substantial physiological impact on ion uptake. This function of cortisol has not been fully appreciated due to an emphasis on the role of cortisol in salt secretion.

In reviewing the evidence prior to 1969, Chester Jones *et al.* (1969) concluded that ''Both ACTH (either directly or by stimu-lating adrenal steroid production) and prolactin act together to maintain water and electrolyte balance of the freshwater fish.'' This conclusion was based on ion flux studies in several teleost species that had been hypophysectomized and/or interrenalectom-ized and had hormone replacement therapy. Evidence collected since that time supports this conclusion and suggests that most of the actions of ACTH are exerted through cortisol (Mayer-Gostan *et al.*, 1987). Fortner and Pickford (1982) found that cortisol increased plasma chloride levels in hypoph-ysectomized black bullhead, and that both prolactin and cortisol were necessary to maintain normal ion homeostatsis under iso-smotic conditions. In hypophysectomized catfish (*Heteropneustes fossilis*) in fresh water, both prolactin and cortisol are necessary to restore plasma sodium and osmolality to the levels observed in intact fish (Parwez and Goswami, 1985). Recent research on hypophysectomized channel catfish (*Ictalurus punctatus*) indicates that prolactin and cortisol interact to restore ion balance in fresh water and isosmotic salinity (Eckert *et al.*, 2001).

Chester Jones *et al.* (1969) hypothesized that ACTH and cortisol may be important in maintaining an active sodium pump whereas prolactin primarily regulates permeability. Although there is substantial evidence indicating that cortisol can increase gill and gut Na^+, $K^+ ATPase$, it is unclear whether this is primarily related to cortisol's seawater-adaptive effects, or whether upregulation of the sodium pump is also involved in ion uptake. If maintenance of the sodium pump is indeed integral to ion uptake, then the effect of cortisol on gill Na^+, $K^+ ATPase$ could be one mechanism by which cortisol exerts a dual osmoregulatory function. Cortisol is also apparently acting to promote the development of the 'freshwater' morphology of chloride cells (Perry *et al.*, 1992). Recent evidence indicates that individual chloride cells of tilapia in fresh water (that are presumably involved in ion uptake) can rapidly develop the morphology characteristic of seawater chloride cells (Hiroi *et al.*, 1999), suggesting that chloride cells are 'bifunctional;' that is, capable of rapidly changing from an ion uptake to an

ion secretion mode. Thus, in the same fashion as described above for the sodium pump, the effect of cortisol to increase the number of chloride cells could act to favor both ion uptake and secretion. More work is required to determine the mechanisms by which cortisol is acting on ion uptake, and the pathways through which cortisol and prolactin interact.

It is important to reconcile the cooperative aspects of prolactin and cortisol on ion uptake with the well-established antagonism of prolactin toward cortisol's induction of salt secretory mechanisms. The *absence of cortisol,* such as through interrenalectomy or hypophysectomy, will result in reduced levels of gill Na^+, $K^+ATPase$ (and perhaps other transporters) that is likely to result in partial or complete loss of capacities for both ion secretion and uptake. In some species (*e.g.,* eels), *increased cortisol levels* may act primarily to promote seawater adapting mechanisms. In other species (*e.g.,* salmonids), cortisol may have the capacity to simultaneously increase both ion uptake and secretory capacity by increasing the number of chloride cells. It is not difficult to imagine that in some marine species, (*e.g.,* seabream) that cortisol acts primarily to promote ion uptake mechanisms (Mancera *et al.*, 1994). During normal acclimation to fresh water or seawater the relative activity of GH and prolactin will also control the direction of cortisol's action in many teleosts. There is great diversity among teleost fishes, and individual species and effector organs will differ in the relative importance of cortisol in controlling ion uptake and secretory mechanisms, and the degree to which cortisol interacts with GH and prolactin.

RAPID REGULATION

In addition to the endocrine control of differentiation of transport epithelia, there is also a more rapid regulatory control of ion transport in teleosts. This area has been the subject of several previous reviews (Foskett *et al.*, 1983; Mayer-Gostan *et al.*, 1987; Marshall, 1995) and only a brief summary and consideration of recent research will be given here. For the most part, evidence of the rapid regulation of ion transport consists of *in vitro* studies examining electrophysiology and ion transport in isolated opercular membranes of seawater-acclimated tilapia and mummichug. The lack of an appropriate *in vitro* model for ion uptake is limiting, and the effects of rapid acting hormones on branchial vasculature can make interpretation of *in vivo* studies difficult. Thus, there is still a limited picture of the hormones involved, and examination of other lines of evidence such as changes in local or circulating levels and receptors is needed to establish a more complete picture of the rapid regulation of ion uptake and secretion. Results to date indicate that the rapid regulation of ion transport in teleosts is multihormonal and complex.

Epinephrine acting through α_2-adrenergic receptors is probably the most physiologically relevant inhibitor of chloride secretion in teleosts (Marshall, 1995). Although early work suggested that cAMP might be acting as a second messenger (Foskett *et al.*, 1983), more recent results indicate that calcium is more likely to mediate this response (Marshall *et al.*, 1993). This represents an unusual regulatory system, since in mammals increased calcium is often associated with increased chloride fluxes, and use of calcium as an intracellular messenger is usually associated with α_1 (and not α_2) receptors. Several other hormones have also been shown to have the *in vitro* capacity to decrease chloride secretion in the opercular membrane, including urotensin II (Marshall and Bern, 1979), acetylcholine (May and Degnan, 1985) and prostaglandin E_2 (Eriksson *et al.*, 1985; Van Praag *et al.*, 1987). Tipsmark and Madsen (2001) have recently shown that elevated gill cAMP levels are associated with inhibition of Na^+, K^+-ATPase in brown trout, though the neuroendocrine signal for this response has yet to be elucidated.

Stimulation of β-adrenergic receptors results in a moderate increase in chloride secretion in isolated opercular membranes from seawater-adapted fish, and is associated with an increase in cAMP (Foskett *et al.*, 1983). The physiological relevance of this response is unclear, however, since the major catecholamine in teleosts is epinephrine, which as discussed above has a pri-

mary role in decreasing chloride secretion. Other hormones may be involved in stimulating chloride uptake through increased cAMP and/or other secondary messengers. Glucagon has also been shown to increase the transepithelial potential (an indicator of chloride secretion) in the isolated gill arch of seawater-acclimated flounder (*Platichthys flesus*) (Davis and Shuttleworth, 1985). Glucagon, Vasoactive Intestinal Peptide (VIP), leukotrienes and Urotensin I have all been shown to effectively increase chloride secretion in the isolated opercular membrane (Foskett *et al.*, 1982*a*; Burgess *et al.*, 1998). The effect of VIP is interesting in light of its physiological relevance in stimulating chloride secretion of the shark rectal gland (Stoff *et al.*, 1979). VIP receptor gene expression has been found in gill, gut, kidney and other tissues of goldfish (Chow, 1997). Angiotensin II has been shown to rapidly increase gill Na^+, K^+ATPase activity in fresh water and seawater adapted eel (Marsigliante *et al.*, 1979). In primary cultures of gill cells from sea bass (*Dicentrarchus labrax*), prostaglandin and arginine vasotocin stimulated short-circuit current and serosal to mucosal chloride transport (Avella *et al.*, 1999). The effect of prostaglandins in this system contrasts with the inhibitory effects noted above, and may be due to the apparent absence of chloride cells in gill cell primary cultures.

There is a similarly complex picture of multihormonal regulation of ion transport in the gut. Epinephrine, neuropeptide Y and somatostatin have been shown to decrease NaCl and water absorption across the isolated intestine of seawater-adapted Japanese eel (*Anguilla japonica*) and goby (*Gillichthys mirabilis*) (Ando and Hara, 1994; Uesaka *et al.*, 1994, 1996; Loretz, 1995). In opposition to these actions, acetylcholine and serotonin inhibit NaCl and water absorption in the intestine of Japanese eel (Ando and Hara, 1994).

There is increasing evidence that atrial natriuretic peptide (ANP) is an important rapid regulator of ion transport in fish (Evans and Takei, 1992). ANP stimulates chloride secretion in the isolated opercular membrane of seawater-acclimated mummichog (Scheide and Zadunaisky, 1988), and

inhibits ion and water absorption in the intestine of winter flounder (*Pseudopleuronectes americanus*) and Japanese eel (O'Grady *et al.*, 1985; Loretz and Takei, 1997). *In vivo*, ANP stimulates Na efflux in seawater-adapted flounder (*Platichthys flesus*) (Arnold-Reed *et al.*, 1991), and urine flow rate and sodium excretion in freshwater-adapted rainbow trout and seawater-adapted toadfish (*Opsanus tao*) (Duff and Olson, 1986; Lee and Malvin, 1987). Kaiya and Takei (1997) found that increased plasma osmolality was more important than volume changes in stimulating circulating levels of ANP in the Japanese eel. Some of the observed actions of ANP are not entirely consistent with a single osmoregulatory action, and a more complete picture of the physiological function of ANP in fish will require more research.

Most of the regulatory actions described above have examined seawater-adapted fish, and there is relatively little information on rapid stimulation of transport epithelia involved in net ion uptake in fresh water (Marshall, 1995). The results of studies on catecholamine control of ion uptake are contradictory: studies using the isolated head found that catecholamines stimulate sodium and chloride influx, whereas *in vivo* approaches found inhibition of ion influx (Mayer-Gostan *et al.*, 1987). The tissue specificity of the effects of epinephrine and norepinephrine on ion transport and the confounding effects of these hormones on hemodynamics of the gill vasculature may partly explain these contradictory results. Oxytocin and urotensin II decreased the short-circuit current and transepithelial potential of the isolated intestine of freshwater-acclimated European eel (Baldisserotto and Mimura, 1997), though the potential contribution of this to ion uptake in fresh water is unclear.

SUMMARY AND PERSPECTIVES

Cortisol has long been known to play an important role in seawater acclimation of teleost fishes. Evidence presented in this review indicates that the GH/IGF-I axis also has a role in seawater acclimation, and that GH, IGF-I and cortisol interact positively to promote salt secretion and the underlying

physiological processes. Prolactin has a well-established role in ion uptake and inhibition of salt secretion. In addition to its role in ion secretion, many studies indicate that cortisol is also involved in ion uptake and can interact positively with prolactin, indicating that cortisol has a dual osmoregulatory function in teleosts. The action of cortisol in promoting ion uptake or secretion may therefore depend in part on the relative activity of growth hormone and prolactin. Under conditions of high growth hormone and low prolactin, cortisol may act primarily to promote salt secretion. Conversely, low growth hormone and high prolactin will cause cortisol to promote ion uptake. The possibility of this latter action in particular requires increased experimental support.

This review has presented evidence that there are many common features to the endocrine control of osmoregulation in teleost fish. It should be noted that only a small number of teleosts have been examined and that we know little or nothing about the hormonal control of osmoregulation in the vast majority of fish. Given the great diversity among teleosts and the differing acclimation responses and strategies that have evolved, it should not be surprising to find that not all teleost fishes will conform to a single scheme. For instance, based on the ability to survive in fresh water following hypophysectomy, there is some indication that prolactin is more important in regulating ion uptake in euryhaline species of marine origin than for those with a fresh water ancestry (Hirano, 1986). One of our research challenges will be to determine how the endocrine control of osmoregulation differs among teleosts, and to what degree it has been shaped by natural selection and reflects the adaptive capacities of species and populations.

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