Prolactin and Osmoregulation

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SYNOPSIS. The actions of prolactin in hydromineral metabolism in all vertebrate classes are reviewed, and the impact of the studies on nonmammalian vertebrates in providing impetus for comparable research in mammals is emphasized. The teleost bladder is a model system for analyzing the interaction of prolactin and cortisol in transport processes; a parallelism is suggested between this model and the prolactin/cortisol regulation of ion and water movements during milk secretion. The *immediate* osmoregulatory effects claimed for prolactin in vitro are questioned; however, under organ-culture conditions the action of the hormone is readily demonstrable. Progress in the isolation of teleost prolactin and the role of osmotic factors in regulating prolactin secretion are briefly described.

INTRODUCTION

By now, it is well accepted that the versatility of prolactin as a vertebrate hormone (Nicoll and Bern, 1971; Nicoll, 1974) is derived to an important extent from the variety of its contributions to osmoregulatory physiology. The discovery that prolactin, "the ... improbable mammalian hormone" (Pickford, 1973), will keep hypophysectomized *Fundulus heteroclitus* alive in fresh water, has led to an enormous and sophisticated literature on the effects of prolactin on teleost fishes. Recognition of the Na^+-retention and water permeability effects of prolactin, especially evident in euryhaline teleosts, has led rather slowly, on the other hand, to the recognition of prolactin's osmoregulatory influence in other vertebrate groups. In representatives of all vertebrate classes, some evidence of a contribution to hydromineral metabolism is now available. This brief survey will refer to the literature of the past few years, delineate some of the newer fields of research, and indicate certain problem areas which

would profit from more investigative effort.

PROLACTIN AND OSMOREGULATION IN CYCLOSTOMES AND ELASMOBRANCHS

In cyclostomes, there is still only a single report (Chester Jones, 1963) which describes increased muscle water content, decreased blood K^+, and markedly decreased muscle electrolytes in *Myxine* held in 60% sea water and treated with prolactin. These actions of prolactin could be of no practical significance if, as it now appears, there is no prolactin in the myxinoid adenohypophysis (Aler et al., 1971; Sage and Bern, 1972). The migrating lamprey may show changed dependence on prolactin in different environments, but no studies along this line have been reported.

In elasmobranchs, information is again scanty. However, Payan and Maetz (1971) were able to rectify the 50% decrease in gill water permeability consequent to hypophysectomy by injections of either prolactin or ACTH. Inasmuch as ACTH, and not prolactin, restored renal function, one can assume that prolactin is not acting through the interrenal. DeVlaming et al. (1974) found that removal of the rostral lobe of the hypophysis (the presumed prolactin-secreting area) resulted in *increases* in plasma urea and sodium, which were reversed by prolactin injections, pos-

sibly indicating increased efflux of these substances or increased water influx. (Conversely, R. Rodgers [personal communication] found decreased urea excretion in two urodele species after prolactin administration.) It is obvious that more work on elasmobranchs is called for, and questions about possible prolactin effects on intestinal absorption of water and ions and on rectal gland function could well be considered.

PROLACTIN AND OSMOREGULATION IN TELEOSTS

Recent reviews (e.g., Lam, 1972; Ensor and Ball, 1972; Utida et al., 1972; Utida and Hirano, 1973; Johnson, 1973) have surveyed the actions of prolactin in teleost osmoregulation. Effects at the branchial, renal, intestinal, bladder, and integumentary levels have been reported. Table 1 summarizes the loci at which prolactin has been implicated in regulating ion and water transport in teleosts. The brief discussion following raises some points derived from recent papers but does not purport to cover the literature even of the '70's.

It is becoming increasingly obvious that no accurate generalization regarding prolactin-dependency for freshwater survival will apply to the Teleostei as a group. Griffiths (1974) has recently analyzed a series of species of *Fundulus* and concludes that the hypophysis is most critical for those species naturally facing extremes of salinity. The bullhead, *Ictalurus melas,* also requires its pituitary or prolactin to survive in fresh water (Chidambaram et al., 1972).

Based on pituitary cytology, Nozaki et al. (1974) consider that prolactin is involved in the ability of newly emerged rainbow trout larvae to withstand the freshwater (hypotonic) environment, as Ichikawa et al. (1973) had earlier suggested for guppy larvae. However, pre-hatching guppy larvae appear to be little affected by prolactin administration (Ichikawa, 1974). Cytological changes were not observed in hatchlings of a marine species *(Hexagrammos otakii)* (Ichikawa, 1973). Sage (1973) concludes that prolactin is involved in both initial and longterm adaptation of *Mugil cephalus* to fresh water. Wendelaar Bonga and Veen-

TABLE 1. *Reported osmoregulatory effects of prolactin in teleosts.*

Branchial

> Decreased Na^+ efflux
> Decreased Na^+, K^+-ATPase
> Decreased water influx (decreased permeability)
> Increased mucus secretion

Intestinal

> Decreased water and NaCl absorption

Renal

> Decreased Na^+ excretion (?)
> Increased glomerular filtration rate
> Increased Na^+, K^+-ATPase
> Glomerular proliferation
> Stimulated tubule cells (cytological)

Urinary bladder

> Decreased water absorption
> Increased Na^+ absorption
> Increased Na^+, K^+-ATPase

Epidermal

> Increased mucus secretion

huis (1974) find that *Gasterosteus* kidney cells are stimulated by prolactin, with an accumulation of particles revealed by freeze-etching on the basal membranes, which appear to be associated with transport processes.

Dharmamba and Maetz (1972) found that hypophysectomized *Tilpia* in fresh water showed increased Na^+ efflux and decreased Na^+ influx; prolactin treatment reduced the efflux, as in other teleosts studied earlier. In *Tilapia* in sea water, Na^+ efflux is also inhibited by prolactin (Dharmamba et al., 1973), and prolactin causes hypernatremia in European eels transferred to sea water (Olivereau and Lemoine, 1973). MacFarlane and Maetz (1974) reported that failure to retain sodium in hypophysectomized flounder in fresh water is due to high branchial efflux, there being no change in the Na^+-absorption pump or in permeability to water. Hypophysectomy did not change water influx in goldfish gills; however, prolactin administration resulted in significant reduction in several teleost species (Ogawa et al., 1973; Ogawa, 1974, 1975).

The teleost integument is becoming increasingly recognized as an osmoregula-

tory organ—or at least as one that responds to prolactin by increased mucus secretion (Olivereau and Lemoine, 1971a, b; Mattheij and Stroband, 1971; Blüm, 1973). Prolactin will also induce an increase in branchial mucus cells in the eel (Olivereau and Olivereau, 1971). The problem of ion transport across teleost skin has not been properly considered, and hormonal control of ion fluxes, apart from mucus secretion, is still an open question.

Salmonids undergoing the parr-smolt transformation should provide excellent material for studying the interaction of prolactin, cortisol, and probably thyroxin in the preparation for seaward migration. Zambrano et al. (1972) have described in a preliminary fashion differences in the prolactin and ACTH cells in *Oncorhynchus masou* between parr and smolt that correlate well with prolactin's role in freshwater adaptation and cortisol's role in seawater adaptation. In *Oncorhynchus nerka* smolts, Leatherland and McKeown (1974) find higher prolactin levels in fish in a hyperosmotic environment than are seen in a hyposmotic environment, suggesting to them greater utilization in the latter. However, they used a heterologous radioimmunoassay system that may be subject to the criticisms raised by Nicoll (1975).

THE TELEOST URINARY BLADDER AS AN OSMOREGULATORY ORGAN

The "newest" target organ on which prolactin has been shown to act is the urinary bladder (Hirano et al., 1971; Johnson et al., 1972), and it is this structure that has particularly intrigued my colleagues during the past 5 years. To some extent, the teleost urinary bladder, an expansion of the fused mesonephric ducts, can be viewed as an extension of the renal system, involved as the collecting duct complex may be, in the selective absorption of water or ions (Foster, 1975). As a sac-like structure, it lends itself to the kind of studies of osmoregulatory membrane physiology that have characterized investigations of the toad urinary bladder, the amphibian integument, the gall bladder, and the everted intestinal sac. The utility of the teleost uri-

nary bladder has also become recognized by investigators who have not been concerned primarily with endocrine regulation (Lahlou and Fossat, 1971; Forster and Danforth, 1972; Hogben et al., 1972; Renfro, 1975; Bayenbach and Kirschner, 1975).

Not all teleosts have permeable bladders, nor do all bladders respond to salinity transfer and to hormone injections. In general, euryhaline marine fishes seem to be the most responsive species (Hirano et al., 1973), but insufficient work has been done to allow conclusions regarding taxonomic position and bladder osmoregulatory properties. In our experience, the bladders of flounders (Pleuronectidae) and of the gobiid *Gillichthys mirabilis* have been particularly useful.

Initially our concept of bladder control (based on studies of flounders) was unihormonal (Utida et al., 1973): prolactin injections simulated freshwater transfer (Johnson et al., 1974). "Prolactin seems to decrease permeability to water while stimulating the neutral ion pump" (Hirano, 1974, 1975). However, it has become increasingly apparent that control is in fact bihormonal: prolactin favoring freshwater permeability (increased ion uptake with greatly reduced water transport) and cortisol favoring seawater permeability (increased water absorption with low ion uptake) (see Johnson, 1973; Doneen and Nagahama, 1973). This "antagonistic" relation has been most convincingly demonstrated in organ-cultured bladders from seawater *Gillichthys*, where cortisol is needed to maintain seawater permeability to water and prolactins reduce this permeability to the freshwater levels (Doneen and Bern, 1974). The organ-cultured bladder is providing a useful model for the study of hormone interaction and also a bioassay system where cortisol-maintained high permeability to water is inhibited by prolactin in a dose-dependent fashion (Doneen, 1974). In addition, these organ-culture experiments establish for the first time a direct, non-organismally mediated, influence of prolactin on a teleost organ.

Functionally oriented ultrastructural studies of the bladder are being pursued in our laboratory (Nagahama et al., 1975).

Such studies raise a new problem in regard to bladder function. The *Gillichthys* bladder epithelium is in fact regionally differentiated: the dorsal, renal zone is lined by a tall epithelium; the remainder (majority) of the bladder is lined by a cuboidal epithelium. It seems possible that these zones subserve different functions. Long-term adaptation to 5% sea water induces activation of the columnar cells, indicated by arrays of elongate mitochondria and extensive tubular smooth endoplasmic reticulum basal to the nucleus. These cells resemble the "chloride cells" of the gill. The presence of more than one cell type in some teleost species has been reported by Gerzeli et al. (1973).

THE QUESTION OF IN VITRO EFFECTS OF PROLACTIN ON OSMOREGULATORY SURFACES

Whereas the organ-cultured teleost urinary bladder can be expected to respond to prolactin (after 2 to 3 days in culture), it should be emphasized that a direct effect on urinary bladders incubated for one to several hours cannot be demonstrated (Johnson et al., 1972). In addition, a direct effect of prolactin on incubated rat jejunal sacs also could not be demonstrated (Ramsey and Bern, 1972; Mainoya, 1975e). Prolactin does not appear to act directly through immediate changes in membrane permeability that involve cyclic nucleotide generation, but rather through longer pathways involving control at the genomic or possibly the post-translational level. Hirano et al. (1973) have reported increased thymidine incorporation by the bladder of the prolactin-treated seawater flounder. Thus, claims of direct effects of prolactin on organs such as the perfused cat kidney (Lockett, 1965), the teleost gill (Ogawa et al., 1973), the toad bladder (Snart and Dalton, 1973; Debnam and Snart, 1975), the avian cloaca (D. M. Ensor, personal communication), or the guinea pig amnion (Holt and Perks, 1975) need to be evaluated carefully. The prolactin may be contaminated with a neurohypophysial octapeptide (see Helbock et al., 1971; Rabkin et al., 1974; Malarkey et al., 1975) or with another osmoactive peptide arising from degradation of the prolactin molecule during purification.

ISOLATION OF TELEOST PROLACTIN

In the earlier studies, we have underlined the difference between piscine and tetrapod prolactins. Most tetrapod prolactins appear in general to replace, at least at higher dose levels, teleost prolactins in teleost systems (Clarke et al., 1973), but the converse is not true. Teleost prolactins do not induce specific lactogenic or cropsac-secretory responses (Nicoll et al., 1966; Nicoll and Bern, 1968). On the other hand, we, and also Chadwick (1966), noted that there was a proliferative effect of teleost pituitary material on the mammary gland, along with occasional stimulation of the cropsac mucosa (significant with *Tilapia* and mullet pituitaries).

Isolation of a teleost prolactin and its sequencing would certainly contribute to our understanding of the molecular requirements for teleost osmoregulation as opposed to the additional requirements for cropsac and mammary responses. Blüm (1973) has purified prolactins from two teleost species and claims that there was no cross-reaction with antibodies to ovine prolactin.

A major collaborative effort between our laboratory and the Hormone Research Laboratory of the University of California, San Francisco, has resulted in the isolation of prolactin from pituitaries of *Tilapia mossambica* collected in Hawaii (Farmer et al., 1974). To date, around 5000 rostral lobes have been extracted, and prolactin has been purified and partially characterized, using a Na^+-retaining assay in *Tilapia mossambica* itself (Clarke, 1973). The amino acid composition of *Tilapia* prolactin resembles that of ovine prolactin, and 20,000 additional *Tilapia* pituitaries are currently being processed. In addition, organ-culture of a small number of *Tilapia* pituitaries under optimal conditions (Nagahama et al., 1975; Nishioka, unpublished) results in the release of large amounts of prolactin, so that ultimately we should be able to compare stored prolactin with secreted prolactin in this species. The *Tilapia* prolactin ap-

pears to be 50 to 100 times more active in *Tilapia* in vivo than ovine prolactin (Clarke, 1973; Farmer et al., 1974).

One side-issue of importance that has emerged from these isolation attempts has been the dissociation of yellowing activity (Sage, 1970; Sage and Bern, 1972) from Na$^+$-retaining activity during fractionation, raising questions about the validity of *Gillichthys* xanthophore-dispersing activity as a bioassay for prolactins.

The purified *Tilapia* prolactin preparation will subserve a variety of purposes. Not only may we be able to characterize the "primary" osmoregulatory portion of the molecule, but also we may be able to distinguish the structural requirements for a "growth" (proliferation) response from those for a "secretory" (specific synthetic) response in tetrapod target organs. The purified protein should provide the antigen needed for the development of a specific radioimmunoassay, allowing the correlation of blood prolactin levels in *Tilapia* with physiological state.

PROLACTIN AND OSMOREGULATION IN AMPHIBIANS

The amphibians, with the need to cope with two environments generally speaking, aquatic and terrestrial, might be expected to call upon prolactin as an aid to adjustment to their hypotonic environment during larval life or, in the case of some urodeles, following second metamorphosis. In the latter instance, prolactin is the waterdrive hormone, and we (Bern and Nicoll, 1968) early speculated on its possible concomitant osmoregulatory role. Nevertheless, the accumulated data are not yet overwhelmingly convincing in regard to an important contribution of prolactin to hydromineral metabolism in amphibians as a class. Crim (1972) could find no evidence for prolactin effects on plasma sodium in *Rana pipiens* or *Taricha torosa*. However, Sampietro and Vercelli (1968) had found prolactin able to correct the hyponatremia of hypophysectomized *Triturus cristatus*, and this has been verified for larval *Ambystoma mexicanum* by Wittouck (1972a) and for

Necturus maculosus by Pang and Sawyer (1974). Prolactin also restores to normal the water permeability of the skin of hypophysectomized *Xenopus* tadpoles (Schultheiss et al., 1972) and increases the ion-absorbing ability of the gills of *Ambystoma* larvae (Wittouck, 1972b). Brown and Brown (1973) report a complex interaction of prolactin and thyroxin on the skin of newts, which could account for observed changes in plasma and urine sodium levels. Earlier Helbock et al. (1971) had demonstrated an effect of human growth hormone (inherently also a prolactin: see Apostolakis, 1968; Forsyth and Folley, 1970) on sodium transport by frog skin.

Further studies of amphibians from additional taxa, from various environments, and at different stages of their life cycle are required to obtain a more meaningful picture of prolactin's possible contributions to water and ion balance in this class. Except for reports of an effect on the toad bladder, to date studies have concerned themselves with possible effects on skin, gills, and kidney. Attention could well be paid to the intestine, and also to a specialized integumentary area, the pelvic "patch" (Baldwin, 1974).

PROLACTIN AND OSMOREGULATION IN REPTILES AND BIRDS

There is still only a single report on prolactin and hydromineral metabolism in reptiles. Chan et al. (1970) reported a synergistic relation between prolactin and corticosterone in restoring normal plasma and tissue composition in the hypophysectomized lizard, *Dipsosaurus dorsalis*. In view of the presence of glands analogous to the avian salt glands in marine reptiles, and the presence of surfaces including the bladder and intestine, all of which could be affected by prolactin, there is room for much more research on representatives of this extremely diverse class of vertebrates.

Information on prolactin and avian osmoregulation is also limited. Peaker et al. (1970—see also Phillips and Ensor, 1972) concluded from experiments on the duck that prolactin may have direct effect on the nasal salt gland (possibly synergistically

with corticosterone—Ensor et al., 1973). Investigation of influences of prolactin on the renal system and intestinal tract (including the cloaca) are obviously called for, and the latter system is presently being investigated in Ensor's laboratory at Liverpool.

PROLACTIN AND OSMOREGULATION IN MAMMALS

In view of the special hydromineral demands upon female mammals during pregnancy and lactation, owing to salt and fluid loss through the placenta to the developing fetus and through the milk to the developing neonate, a consideration of possible effects of prolactin other than those obviously related to sustaining gestation and lactation seems justified. During these periods circulating prolactin levels would be expected to be high. Because much of the impetus for investigating the possible effect of prolactin on mammalian osmoregulation, including in man, has been derived from work on nonmammalian vertebrates, especially teleosts (see Horrobin, 1973), a section on mammals is included in this survey.

Fetal osmoregulation

As has been pointed out earlier (Bern, 1972), the mammalian fetus is potentially exposed to prolactins from several sources: the placenta (see Talamantes, 1975), the maternal pituitary, and the fetal pituitary. Prolactin levels in human amniotic fluid are high (see Ben-David and Chrambach, 1974) and suggest that prolactin could contribute to the solution of osmoregulatory problems faced by the mammalian embryo during its aquatic existence in amniotic fluid (see Friesen and Hwang, 1973). Prolactin could affect the embryo itself, in analogy with the aquatic teleost fish, at the levels of its intestine, its kidney system, and even its transitory gills. Even more likely could be effects on extraembryonic membranes, including the amnion and the chorion, and the yolksac and allantois where these are developed as sacs. Indeed, where complex placental structure exists, the hormonal regulation of placental transport remains an uninvestigated question. Obviously, regulation of transport across ex-

traembryonic membranes applies to the other amniote classes as well (birds and reptiles). With the important discovery of the effect of fetal arginine vasotocin on transport by the amniotic membrane in the guinea pig (Vizsolyi and Perks, 1974), reality can begin to replace speculation in this area. Investigations of influences of prolactin on extraembryonic membranes are currently under way in Vancouver (Holt and Perks, 1975) and in Berkeley (by B. Stehle). The fetal urinary bladder and skin would also appear to be appropriate organs for study of possible prolactin effects (France et al., 1974; France, 1974).

Intestinal absorption

In addition to a contribution to fetal osmoregulatory physiology, prolactin also has significant effects on transport of water and ions by the mammalian intestine. Following up on an initial report (Ramsey and Bern, 1972), Mainoya has made a detailed analysis of the effects of prolactin on intestinal transport (Mainoya et al., 1974; Mainoya, 1975a,b,c,d,e). Prolactins (including human placental lactogen) will cause increased fluid and ion uptake by the rat jejunum; the increased transport of fluid is Na^+-dependent, and the ion uptake is glucose-dependent. Increased absorption of glucose and of amino acids has also been demonstrated, and is Na^+-dependent. The increase in water and solute influx, then, may not in fact be primarily of osmoregulatory significance, but rather coupled to more efficacious absorption of the products of digestion. Nevertheless, the overall effect is to increase the rapidity of uptake fluid and ions in rats subjected to increased prolactin levels. Shulkes et al. (1972) found that prolactin injection led to increased NaCl intake in wild rabbits and feel, as does Mainoya (1975d), that during lactation the hormonal milieu is responsible for the increased salt appetite.

Renal effects

A renal effect of prolactin in mammals, including man, has been indicated for some time, so that effects on the kidney also need

to be considered during gestation and lactation. Increased sodium retention following administration of human growth hormone (inherently a prolactin) was reported in human subjects (Biglieri et al., 1961), and Frantz et al. (1972) noted a frequent association of renal failure with high human prolactin levels (as if the latter were attempting to compensate for the osmotic disturbance). However, Friesen and Hwang (1973) question any consistent correlation between high human prolactin levels and sodium retention. Administration of ovine prolactin to human subjects resulted in Na^+ retention, according to Horrobin et al. (1971). Lockett and Nail (1965) had earlier reported Na^+-retention following prolactin treatment of rats.

In a series of studies, Horrobin and his colleagues have presented evidence for the ability of prolactin to counter saluretic effects of progesterone in pregnant and pseudopregnant rabbits (Lloyd et al., 1971) and of aldosterone in the presence of increased salt intake (Burstyn et al., 1972) or in the presence of excess cortisol (Horrobin et al., 1973a) in Merino ewes. Prolactin also restores the normal antidiuretic action of arginine vasopressin in ewes in the presence of excess cortisol (Horrobin et al., 1973b).

Milk secretion

Another area wherein prolactin may be considered from an osmoregulatory viewpoint in mammals is in the process of milk secretion itself. Only recently has significant attention been paid to the fact that the mammary epithelial cells, concerned as they are with the transport of fluid, ions, and other osmoactive molecules, in fact form surfaces where the problems of paracellular movements (nature of junctions) and transcellular movements (ion pumps, altered membrane permeabilities) are similar to those faced by absorbing and excreting surfaces generally (Mackenzie and Lascelles, 1965; Linzell and Peaker, 1971, 1973; Pitelka et al., 1973; Peaker and Linzell, 1973).

Normal and tumorous mammary epithelia (and other epithelia) form "domes" in monolayer cell cultures (Leighton et al., 1969; McGrath, 1971; Enami et al., 1973; Hosick and Nandi, 1974), which accumulate fluid in a pulsatile fashion (Visser and Prop, 1974). These "hemi-alveoli" (Leighton's "hemi-cysts") can serve as excellent models for the examination of water and electrolyte transport mechanisms. In addition, Misfeldt et al. (1974) have succeeded in producing artificial membranes of renal tumor epithelial cells by a method which is also applicable to mammary epithelia (Hamamoto et al., 1974).

Na^+, K^+-ATPase is an enzyme commonly associated with cells engaged in ion-pumping; in certain teleost systems (branchial, renal, intestinal, and/or urinary bladder) it has been shown to be responsive to prolactin (Pickford et al., 1970a; Kamiya 1972; Utida et al., 1974), as well as to cortisol (Pickford et al., 1970b; Milne et al., 1971; Epstein et al., 1971; Butler and Carmichael, 1972). The enzyme also occurs in the mammary gland (Kimura, 1969; Misfeldt et al., 1970; Vreeswijk et al., 1973), which should not be surprising if active monovalent cation transport occurs. It would be of interest to determine any specific relation between prolactin and corticosteroid—the lactogenic hormones—and activity of this enzyme in mammary cells. It is interesting to recall that the primary osmoregulatory hormones in teleosts are also prolactin and cortisol and to suggest that studies on teleost systems may have relevance to studies of milk secretion in mammals (see Fig. 1).

Prolactin is one of the few hormones about which even conjecture as to mode of action is minimal. In view of the recent observations in regard to thyroxin action and active Na^+ transport (see Ismail-Beigi and Edelman, 1974), examination of the role of ion pumps and membrane permeability alteration in the response of epithelial cells to prolactin may prove profitable (c.f. Falconer and Rowe, 1975).

Prolactin effects on other secreting epithelia (mammalian prostate, catfish seminal vesicle) could also involve electrolyte and fluid transport mechanisms, and there may yet emerge a commonality of action on
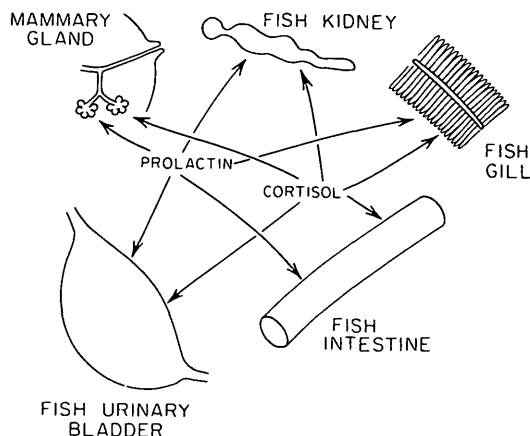
FIG. 1. Prolactin and cortisol are the primary osmoregulatory hormones affecting various water- and ion-transporting structures in teleosts. They are also the lactogenic hormones in mammals, regulating the transport of water, ions and other osmoactive molecules across the mammary epithelium, in addition to the synthesis of other milk consitiuents.

secretory organs involved in synthesis and release of a variety of secretory products. The speculative theme underlying this brief discussion is an attempt to relate the osmoregulatory activity of prolactin with its several secretion-stimulatory activities.

OSMOTIC FACTORS AND PITUITARY PROLACTIN SECRETION

This survey will not attempt to review recent work on the cytophysiology of the prolactin cells in fishes, or in other vertebrates, in relation to osmotic alterations (see Schreibman and Holtzman, 1975). Nor will it present any detailed treatment of the control of prolactin secretion and the role of osmotic factors in this process. Nagahama et al. (1975) have recently reviewed in some depth the control of prolactin secretion in teleosts and the direct and possibly indirect influence of blood osmotic pressure. Suffice it to say that in this area also, the work on teleost fish osmoregulation has apparently led to investigations on mammals, including man, of the relationship between blood osmotic pressure and plasma prolactin levels.

In birds (ducks and gulls) Ensor and Phillips (1970, 1972) have noted important decreases in pituitary prolactin levels in response to salt-loading or dehydration, most notably where the osmotic stress was greatest.

Relkin and Adachi (1973) reported increased pituitary and plasma prolactin levels in the rat deprived of sodium. However, later Relkin (1974), in the rat, and Buckman and Peake (1973), in man, found direct correlation: hyperosmolality of the blood resulted in high prolactin levels. La-Bella et al. (1974) report that there was an inverse relationship between release of prolactin from bovine pituitaries in vitro and the osmolarity of the medium, extending the fundamental observation of Sage (1968) in teleosts regarding the sensitivity of prolactin cells themselves to decreased Na^+ concentration (see Zambrano et al., 1974; Nagahama et al., 1975). Any generalization regarding the sensitivity of prolactin synthetic and release mechanisms to osmotic factors requires an intensification of investigation of the nature of the phenomenon throughout the vertebrate subphylum. Attention should be called again to the point first emphasized by Olsson (1968) regarding the ontogeny of the rostral pars distalis containing the prolactin cells and its initial, and occasionally persistent, connection in teleosts with the buccal cavity and hence with the aquatic environment.

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