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REGULATION OF PIGEON CROPMILK SECRETION AND PARENTAL BEHAVIORS BY PROLACTIN

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CONTENTS

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ABSTRACT

Prolactin stimulates the growth and development of specialized epithelial cells lining the cropsac of pigeons and doves (family *Columbidae*), leading to formation of "cropmilk," which is fed to the newly hatched squab. This system of milk feeding is unique among birds. To support the feeding of cropmilk, a complex array of behavioral adaptations are also supported by high levels of prolactin secretion in columbids during parenting. These specializations include elevated food intake (hyperphagia), nest attendance, and regurgitation feeding of the squab. Although prolactin is clearly important for these behavioral adaptations, the precise physiological and mechanistic bases for these behavioral effects remain controversial. The molecular mechanisms of prolactin action in the cropsac epithelium have been studied by cloning prolactin-induced genes, by cloning and expressing the pigeon prolactin receptor, and by analyzing the transcription factors that are activated after prolactin treatment. The avian (pigeon) prolactin receptor is a member of the cytokine receptor superfamily and uniquely contains a complete duplication of the extracellular ligand-binding domain. One of the early signal-transducing actions of prolactin in cropsac epithelium is the activation of signal transducer and activator of transcription (STAT) proteins via tyrosine phosphorylation. This fundamental signaling pathway is shared with mammalian prolactin target tissues. The convergent evolution of milk feeding and the behaviors that support parenting in columbids and mammals has depended on adaptation of both conserved mechanisms and divergent physiological processes.

INTRODUCTION

The cropsac of pigeons and doves is uniquely adapted to produce milk that is fed to the offspring. The extraordinary growth of the newly hatched birds attests to the nutritive value of the milk. This adaptation is likely to be one of the main ingredients in the pandemic success of pigeons and other members of the dove family, which inhabit nearly all parts of the world. The role of prolactin in this process goes beyond direct stimulation of the cropsac itself and includes regulation of parental feeding behaviors, growth of the gut (splanchnomegaly), and general changes in metabolism (86). In this paper, we examine the current state of knowledge about this fascinating biological system. We focus on two aspects that have recently been the subject of substantial research: (*a*) the role of prolactin in the integration of parental behaviors that support milk and seed feeding to the young and (*b*) the molecular basis of prolactin's direct actions on the cropsac epithelium.

THE NATURE OF CROPMILK

Unlike the complex developmental and hormonal architecture underlying mammalian milk secretion, the cropmilk system in pigeons is relatively simple. The cropsac is an outpouching of the esophagus, and the histoarchitecture of the cropsac epithelium resembles most closely the epithelium of normal esophagus. The cropsac epithelium grows as a simple squamous layer approximately 10 cells thick from a proliferating basal zone (stratum basale), and the cells undergo differentiation as they move away from the basement membrane toward the lumen. The differentiating layer (stratum spinosum) is the region of formation of the cropmilk cell type. Normally, the epithelial cells slough off the lumenal surface in the course of cell replenishment. Stimulation of the tissue by prolactin leads to storage of large amounts of lipid and protein as well as the sloughing of cells in masses that have a cheese-like texture. This "holocrine" (whole-cell) secretion is then fed to the young pigeon squab. In concert with altered cell differentiation in the stratum spinosum, the stratum basale is mitogenically stimulated, resulting in thickening, by as much as 10-fold, of the epithelium.

The nutritional composition of cropmilk was analyzed in several early studies. In contrast to mammalian milk, cropmilk has no detectable carbohydrate (21, 36). Protein content is approximately 60% of the dry weight, and the bulk of the remainder is lipid (21, 26). The fats in cropmilk are almost exclusively medium-chain unsaturated triglycerides (36), as in most mammalian milks. The ash (mineral) content of cropmilk is very similar to that of whole cow's milk (~5–6%) (21, 26). No evidence of casein-like proteins, which sequester calcium in mammalian milk, is apparent, although one of the major prolactin-induced genes in cropsac is an isoform of the calcium-binding protein annexin I (60). Regulation of this protein is discussed extensively in subsequent sections.

The time during which squab feed exclusively on cropmilk is relatively short. After hatching, the parents provide only cropmilk for the first three days or so (Figure 1). Thereafter, they provide a mixture consisting of cropmilk and increasing amounts of seeds and other food materials. During the initial two days, the body weight of the squab doubles because it is gorged with cropmilk by both parents. This rapid increase of body size in an altricial (immature) nestling provides evidence for the physiological and evolutionary importance of cropmilk. Although both pigeons and mammals feed milk to their offspring, the complexities of diet over the long term are less important in pigeons than in mammals because mammalian milk is the exclusive source of nutrients for a much longer period of infant life. In addition, some of the factors supplied to the mammalian offspring in milk, such as immunoglobulins, are provided via the yolk in birds. Therefore, a simple system for producing large masses

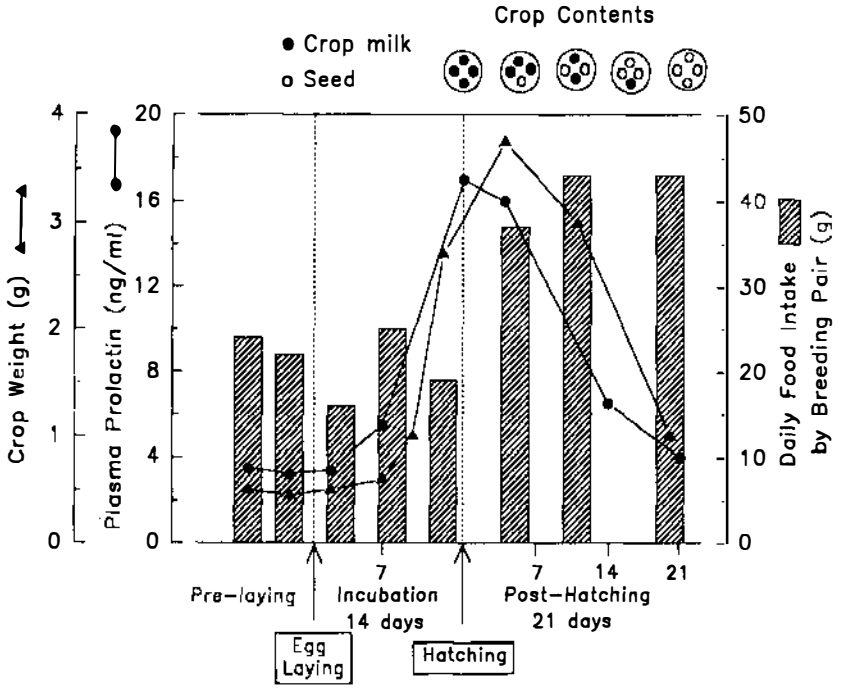

Figure 1 Changes in plasma prolactin concentration, cropsac development, crop content, and parental hyperphagia during the ring dove breeding cycle. The patterns of changes depicted for plasma prolactin and crop weight are typical of both sexes, although females tend to exhibit higher peak values of both parameters than males. (Data are summarized from numerous sources; see text for citations.)

of protein- and fat-laden cells that can be easily fed to the squab is an exceptionally efficient adaptation for this group of birds. To support the production and feeding of cropmilk, parents must exhibit appropriate behaviors during the incubation and brooding phases. These behavioral adaptations are the subject of later sections of this review.

STRUCTURE OF THE CROPSAC

Gross Morphology

Most birds and many reptiles have a crop organ, which is an expansion of the lower esophagus leading to either a unilateral or bilateral pouch. This pouch is used to store food, which passes in a controlled fashion into the proventriculus (glandular stomach) and gizzard for grinding and digestion. In this manner, the crop extends the period of food availability and allows large masses of

food to be taken in at optimal mealtimes. In pigeons and doves, the crop is bilobed. In addition to the foregoing functions, it is designed to produce cropmilk. Most features of its anatomy are unremarkable. It is a delicate, membranous pouch in the unstimulated birds. A thin muscularis (longitudinal and transverse smooth-muscle fibers) and a connective tissue lamina propria underlie the mucosal epithelium.

Microscopic Anatomy

Dumont studied the cytology of the cropsac epithelium and the changes induced during brooding by prolactin (36). He observed an increase in mitoses in the germinal layer (stratum basale) and a consequent thickening of the epithelium in response to prolactin. In the differentiating layer (stratum spinosum), the cells in the unstimulated birds are relatively flattened and unremarkable. After prolactin stimulation, however, these cells develop large lipid globules, dense arrays of polysomes, and a more active endocytotic apparatus (36). Figure 2 shows the cytology of prolactin-stimulated cropsac epithelial cells. Note that the cropsac epithelium has little or no rough endoplasmic reticulum even when hormonally treated, which is consistent with the fact that the cropmilk is comprised of whole desqaumated cells, not of an extracellularly secreted fluid.

Whereas the exocytotic apparatus is minimal, the endocytotic system is highly active in stimulated cropsac epithelium. Several types of primary endosomes can be distinguished (36), and multivesicular bodies are common. The fats stored in cropmilk lipid globules are not locally synthesized but rather are taken up by endocytosis and stored (64). This represents another difference between pigeon and mammalian milk production. In mammals, the milk fats are synthesized de novo in the alveolar epithelium.

HORMONAL CONTROL OF CROPSAC DEVELOPMENT

The first pituitary hormone to be purified was the pigeon cropsac-stimulating factor, termed prolactin by Riddle and his coworkers (102). In a remarkable series of papers in the late 1920s and early 1930s, Riddle's research group elaborated on the basic physiology and biochemistry of prolactin and established the paradigm for future purification of the remaining pituitary hormones.

Both male and female pigeons and doves produce cropmilk during brooding, respond to exogenous prolactin, and feed their squab. No qualitative or quantitative differences in cropmilk have been demonstrated between males and females, or in gonadectomized animals. Therefore, the reproductive hormones do not play any discernible role in cropsac growth and development. Likewise, thyroid hormone, adrenal steroids, growth hormone, the gonadotropins, and other pitutary hormones seem to play no direct role in cropsac

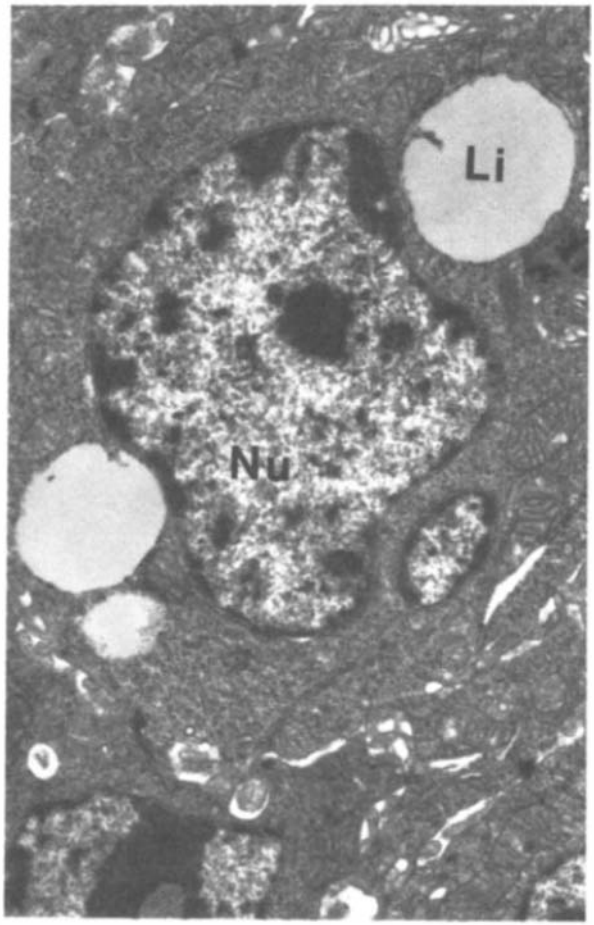

Figure 2 Ultrastructure of prolactin-stimulated cropsac epithelial cells. Cropsac epithelial tissue, stimulated by hormone injection, was prepared by standard osmium fixation and epon embedding and photographed at a magnification of ~15,000×. The nucleus (Nu) and lipid globules (Li) are marked. Note the lack of rough endoplasmic reticulum, the small amounts of Golgi apparatus, and the numerous vesicles and membrane invaginations indicative of endocytotic activity.

stimulation (108). Even before weaning, the pigeon squab can respond to prolactin with cropsac growth and differentiation (59). In adults, prolactin levels rise during the latter half of incubation, and cropsac development directly follows increased prolactin secretion (Figure 1) in the pigeon as well as in the ring dove.

THE PIGEON PROLACTIN RECEPTOR

The cropsac prolactin receptor has been studied using binding assays (43, 70, 109, 110). Its cDNA was recently cloned. Its nucleotide sequence was determined and the protein characterized by expression of the recombinant protein in *Escherichia coli* (23). The first evidence of prolactin receptors in cropsac was the observation that the epithelium specifically sequestered radiolabeled mammalian prolactin in vivo (110). Cropsac mucosal microsomes bound prolactin with high affinity ($7 \times 10^{-10}\ M^{-1}$), and the potency of various mammalian prolactins was directly related to their ability to compete with labeled hormone for binding to receptor (43). Prolactin injections increased receptor content (109).

The full-length cDNA encoding the pigeon prolactin receptor was cloned by screening cDNA libraries and by using reverse transcription coupled to the polymerase chain reaction (PCR) (23). This receptor cDNA encodes a deduced protein of 830 amino acids with two highly hydrophobic domains: an N-terminal secretory signal and an internal putative membrane-spanning domain. The pigeon prolactin receptor, like that of mammals, is characterized by features that place it in the cytokine receptor family (65). The first known example of this shared receptor style was the growth hormone receptor (85). The receptors for prolactin, erythropoietin, interleukins (except IL-1 and IL-8), granulocyte-macrophage colony–stimulating factor, leukemia inhibitory factor, ciliary neurotrophic factor, and other cytokines share significant homologies in the extracellular and, to a lesser extent, intracellular domains (4, 62, 65, 67). The diagnostic features of the cytokine receptor family are two pairs of conserved cysteines and a tryptophane-serine-any residue-tyrptophane-serine (WSXWS) motif in the extracellular domain. In addition, most members of the family share a proline-rich motif (PRM) in the membrane-proximal intracellular region (98). The functions of these conserved sequences are not fully understood. The cysteine pairs in the extracellular domain probably enable protein folding to produce the ligand-binding domain, and the WSXWS motif is involved in protein-protein interactions and receptor internalization (65).

The avian prolactin receptor, unlike that in mammals, contains two complete units of the extracellular cytokine homology domain (23, 119). Both pigeon and chicken receptors have this duplication, which has been referred to as a double antenna structure (119). Although other members of the cytokine receptor family (e.g. IL-3Rβ chain) have a duplicated extracellular domain, none retains all of the characteristic homologous motifs. In contrast, the avian prolactin receptors have all of the homology domains in both extracellular repeats. The similarity of the two extracellular repeats of the pigeon prolactin receptor (64%) is practically equivalent to the similarity of the membrane-

proximal unit to the extracellular domain of mammalian receptors (65%) (23). This observation suggests that the duplication of the extracellular domain occurred at about the same time as the divergence of the avian and mammalian lineages (~250 million years ago).

To address the functional role of the duplicated extracellular domains, both the wild-type pigeon receptor and a truncation mutant encoding only the membrane-proximal repeat and intracellular domain were expressed in mammalian cells and used for binding studies. The truncated receptor form was indistinguishable in terms of both hormone specificity and binding affinity when mammalian hormones were used (23). In addition, cotransfection of the full-length and truncated pigeon prolactin receptors with a β-casein gene promoter/reporter plasmid resulted in equivalent induction of gene transcription in the Chinese hamster ovary (CHO) cell line (X Chen, H Buteau, M Edery & N Horseman, unpublished data). In the context of expression in vivo, the duplicated extracellular domains of the avian prolactin receptors may be physiologically important. However, in the cell culture system they do not appear to play a fundamental role in the synthesis of the receptor, in binding to hormones, or in signaling.

GENE EXPRESSION IN CROPSAC

Prolactin-Induced Genes

The genes that are preferentially induced in cropsac by prolactin include those that encode specific intermediate filament proteins (CMP 58 and CMP 50.5) associated with lipid globule storage (64) as well as those encoding an unidentified 25-kDa protein (100), ornithine decarboxylase (63), lipoprotein lipase (49), and annexin I_{cp35} ($anxI_{cp35}$) (60, 61, 101). The expression of CMP 58 and 50.5 was increased approximately threefold by prolactin (64). These proteins are closely associated with the growth of lipid globules in the cropsac epithelium. They appear to form a cage-like matrix surrounding the lipid globules (Figure 2), which are not membrane bound (64). Similarly, vimentin, an intermediate filament protein commonly found in fibroblastic cells, forms a cage surrounding lipid globules during adipocyte differentiation (44). Two features distinguish lipid storage in adipocytes from that in cropsac epithelium: the specialized membrane that surrounds adipocyte lipid globules, and the local synthesis in some mammals of at least some of the lipid stored in adipocytes.

The $anxI_{cp35}$ mRNA is the most abundant of the prolactin-induced gene products in cropsac (Figure 3). It encodes a specialized form of annexin I that lacks regulatory phosphorylation sites, which are characteristic of other annexin I proteins (53, 61, 114). The gene for $anxI_{cp35}$ covers 15 kilobases and 13 exons (57). The pigeon, unlike other species, has two separate annexin I

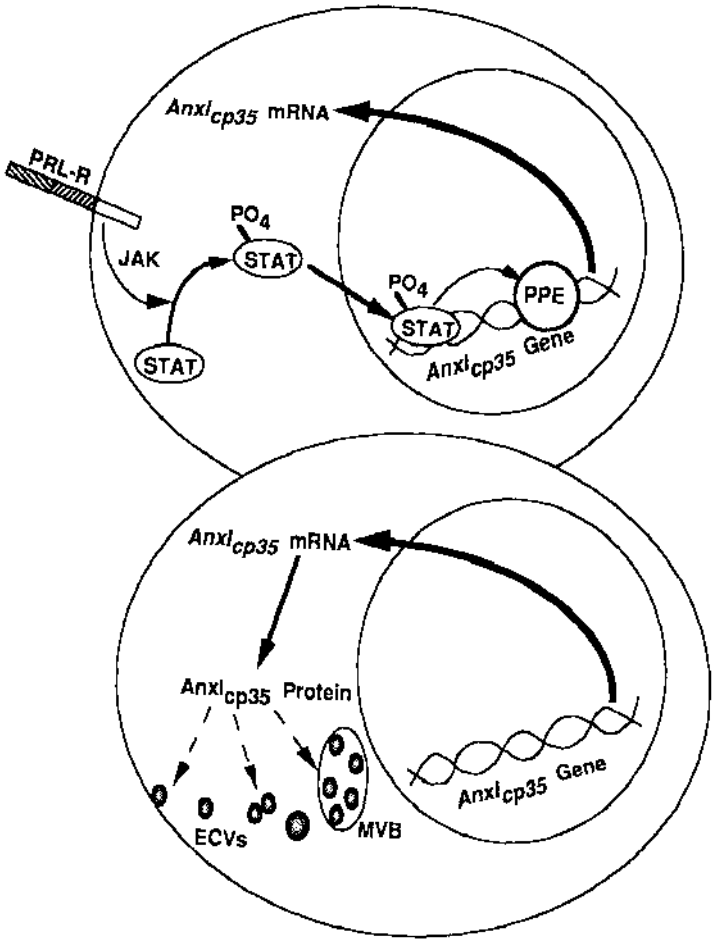

Figure 3 The regulation of cropsac epithelial cell gene expression. In the upper diagram, the regulation of *annexinI$_{cp35}$* gene expression is depicted schematically. The unique avian prolactin receptor (PRL-R) includes two repeats of the extracellular ligand-binding domain. Prolactin binding to its receptor results in phosphorylation of one or more signal transducer and activator of transcription (STAT) proteins, presumably through the activation of a Janus kinase (JAK). Upon activation, STAT is translocated to the nucleus, where it interacts with sites in the *anxI$_{cp35}$* gene and presumably with sites in other nuclear genes as well. A separate promoter-proximal element (PPE) is partly responsible for transcriptional activation, and the STAT site and the PPE may interact. In the lower diagram, *anxI$_{cp35}$* mRNA translation leads to synthesis of the cp35 isoform of annexin I protein. Indirect evidence suggests that cp35 participates in the formation and traffic of endocytotic vesicles (ECVs) and multivesicular bodies (MVBs).

genes (48). One of those genes (*anxI$_{cp37}$*) is constitutively expressed in multiple tissues, and the other (*anxI$_{cp35}$*) is exclusively expressed in the cropsac under prolactin regulation (48). The biochemistry of annexin I, together with data suggesting that annexins may be involved in endosomal function (38, 46), indicates that the anxI$_{cp35}$ protein may play an important role in the stimulation

of endocytosis by prolactin (59). It may also sequester calcium so as to provide adequate amounts of this nutrient to the squab. The transcription of $anxI_{cp35}$ increases rapidly after prolactin treatment, and a significantly elevated mRNA level was detectable by northern blot analysis within 2 h of hormone treatment (101).

Transcription Factor Regulation

The *cis*-acting DNA elements and associated *trans*-acting factors of the $anxI_{cp35}$ gene have been studied. Two elements that interact with specific hormone-dependent DNA-binding proteins have been examined (Figure 3): (*a*) a promoter-proximal region, and (*b*) a distal element that binds a prolactin-induced STAT protein (114, 128). Using in vitro transcription, Xu & Horseman (128) showed that the promoter-proximal *cp35* gene element (PPE) mediates a marked stimulation. Addition of crude nuclear extract from prolactin-stimulated cells markedly enhanced transcription from the *cp35* promoter in HeLa cell extracts but had no effect on a control gene promoter. Extracts from unstimulated cells had no stimulatory activity. The sequence between –32 and –72 bp (upstream of the transcription start site) bound at least two proteins (128), one of which has a molecular weight of 118,000, as determined by southwestern blotting (Xu & Horseman, unpublished data). The p118 factor appeared within 1 h of stimulation with prolactin, and its activity was suppressed by treating the extracts with phosphatase. Other proteins interacting with the promoter-proximal region were stimulated by prolactin with slower kinetics, indicating an orderly assembly of multiple proteins on the proximal element, which ultimately results in stimulation of transcription activity. The sequential appearance of multiple DNA-binding proteins for the proximal element suggests that these factors may have multiple levels of regulation. For example, latent factors may be activated rapidly, whereas other factors may be synthesized later de novo.

The PPE that mediates part of the prolactin transcriptional effect contains a complex set of nested dyad symmetries, $^{-45}$CACTCGTGCAGTG^{-33}. The symmetrical bases in this element are similar to those in the "E box" (5′-CAN$^{2-4}$TG), a consensus site that binds basic helix-loop-helix (bHLH) proteins (7, 94). E12 and E47, which are ubiquitous bHLH proteins, can bind to sequences equivalent to the cp35 dyadic element (CANNNNTG) and form homo- and heterodimers with other bHLH proteins through leucine-zipper motifs (50, 118). The factors that bind to this E box–like element in the *cp35* gene are currently being cloned to determine whether they represent members of the bHLH family, which are regulated by prolactin.

The distal element was identified by sequence comparison with elements known to mediate interferon (IFN)–γ responses. These elements are termed gamma-activated sequences (GAS) (68, 106, 112), and sites with similar se-

quences are present in the promoter elements of the genes of several mammary gland milk proteins (107, 125). The distal *cp35* GAS-like site (termed GLS44) is located at −1144 bases upstream of the transcription start site. Its sequence is 5′-TTTCAGTAA. Protein-DNA binding measurements using the gel-electrophoretic-mobility-shift assay showed a set of transient prolactin-induced protein-DNA complexes that appeared within 10 min of hormone administration and peaked at 30 min. A related sequence from the human c-*fos* gene, containing the sequence 5′-TTCCTGTAA, also bound a prolactin-responsive protein that was simultaneously induced, but the two sequences did not compete for protein binding. This result indicates that multiple prolactin-responsive proteins are simultaneously induced and have different nuclear targets. Binding of the prolactin-induced nuclear factor to this GLS44 sequence was specifically inhibited by antibodies to mammalian STAT1α (113). A set of 95- and 70-kDa cropsac proteins, which immunoreacted with anti-STAT1α, were transiently phosphorylated on tyrosine in response to prolactin (114). Therefore, the p95 and/or p70 proteins are prolactin-induced STAT-family factors that may play a role in the initial activation of multiple genes in cropsac epithelia or in other prolactin target tissues.

STAT5 (mammary gland factor) is an important factor in the regulation of the β-casein gene of the rat (124). The activity of this factor (as indicated by DNA binding) is enhanced specifically by prolactin (126). Cotransfection of STAT5 with prolactin receptors leads to prolactin-dependent activation of the casein gene promoter. Therefore, activation of STAT-family transcription factors is a mechanism of prolactin signaling common to both birds and mammals.

Prolactin acts via multiple transcription regulatory pathways to induce specific gene expression and mitogenesis in cropsac. The sharing of fundamental properties of these signaling systems across phylogenetic lines and in multiple tissues is consistent with the many roles of prolactin in vertebrate reproduction and perinatal physiology.

PROLACTIN AND PARENTAL FEEDING OF THE SQUAB

Prolactin has long been implicated in the expression of a variety of behaviors associated with parental care in birds (see 9 for review). Prolactin and gonadal steroids are essential for the induction of incubation behavior in the domestic turkey (37). Moreover, prolactin appears to act directly on the central nervous system to induce this behavior (129). In ring doves, the involvement of prolactin in the induction of incubation behavior is unclear (81), although some data suggest that prolactin can maintain incubation once induced (79) and can sustain an established readiness to sit on eggs whenever incubation is interrupted by separating the birds from their nests (66, 82). These behavioral patterns are consistent with the pattern of prolactin secretion observed during

the reproductive cycle of ring doves. Whereas in galliform species such as turkeys and chickens, plasma prolactin begins to rise at egg laying and remains elevated throughout incubation (see 74 for review), in ring doves plasma prolactin does not exhibit a sustained increase until midincubation (Figure 1), and this hormone does not reach peak levels until hatching has occurred (24, 51, 79). This finding implies that in *Columbidae,* prolactin may have more to do with parental activities associated with care of young than with the incubation of eggs.

In ring doves, prolactin not only promotes crop development and cropmilk formation, but also facilitates the regurgitation behavior that the parents must exhibit in order to transfer the cropmilk to the offspring. In a process analogous to suckling-induced prolactin release in mammals, stimuli associated with or generated by parental regurgitation behavior in turn feed back to promote additional prolactin secretion (13). Lehrman (80) initially reported that more than 80% of the isolated, nonbreeding ring doves given subcutaneous or intradermal injections of prolactin fed foster squab that were presented to them, whereas none of the control subjects did so. However, prolactin was only effective if the birds had previous breeding experience. A subsequent study confirmed the stimulating effect of prolactin on regurgitation behavior in experienced birds (59; Figure 4). In inexperienced birds, Lott & Comerford (88) showed that neither prolactin nor progesterone alone stimulated parental regurgitation; a combination of the two was effective.

Prolactin-induced parental regurgitation is greatly reduced in nonbreeding, experienced doves with anesthetized crops (80). This finding suggests that prolactin indirectly facilitates the display of regurgitation behavior by promoting crop engorgement and thus altering the sensitivity of the crop and the overlying skin to tactile stimuli from the begging squab. However, multiple sites of prolactin action are likely because 33% of the prolactin-treated birds with anesthetized crops fed the squab. Later studies revealed that breeding doves would feed foster squab introduced during early stages of incubation before significant crop development and engorgement had occurred (55, 56, 71). Evidence for a central site of prolactin action in promoting feeding activity was suggested by a recent study in which experienced doves were tested for parental responsiveness following ICV injections of ovine PRL at doses too low to promote cropsac development (12). Birds in this study were food deprived before the test to eliminate the possibility that increased food consumption and the resulting crop engorgement contributed to the response. The incidence of parental regurgitation in these doves was somewhat lower than that induced by systemic injection of prolactin but was still significantly higher than that of control birds (Figure 4). Importantly, the prolactin-injected birds showed a marked increase in the incidence of parental feeding invitations that did not culminate in actual regurgitation activity (Figure 4). This increase in

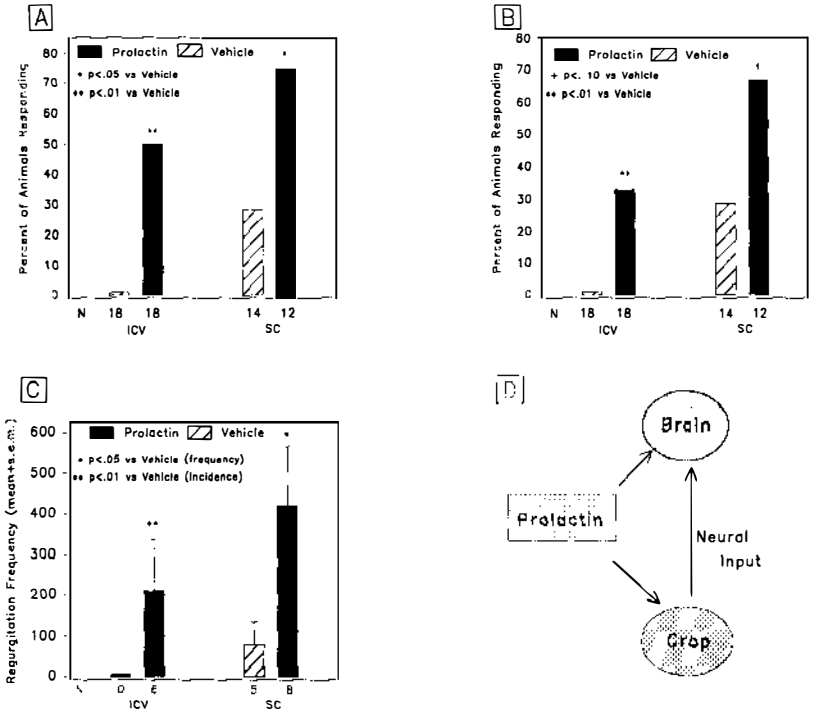

Figure 4 Differences in the effectiveness of central (intracerebroventricular, ICV) and peripheral (subcutaneous) administration of ovine prolactin in promoting parental regurgitation feeding behavior (Panels B and C) and feeding invitations (Panel A) in nonbreeding doves with previous breeding experience. Each bird was housed in isolation and given injections of prolactin or vehicle twice daily for 8 consecutive days, followed by a 2.5-h parental behavior test with a hungry 6–8-day-old foster squab. The low dose of ovine prolactin used in the ICV group (2 µg/day) did not induce detectable cropsac growth. ICV-injected birds were also food deprived prior to the test in order to minimize the potential effect of crop distension on parental activity. Each group consisted of equal numbers of males and females, and no differences in parental behavior were observed between the sexes. Panel D depicts a dual-site model of prolactin action in facilitating parental regurgitation feeding activity. In this model, prolactin exerts direct effects on the neural substrate(s) for parental behavior or enhances the effectiveness of the stimuli from the squab that normally elicit parental activity. Prolactin is also hypothesized to act indirectly to facilitate parental behavior by inducing crop growth and engorgement with cropmilk. This engorgement and distension may in turn facilitate parental regurgitation by generating proprioceptive input to the brain. (Data are from Ref. 12.)

feeding attempts indicates that the prolactin-treated birds exhibited adequate parental motivation, but perhaps because their cropsacs were neither developed nor engorged, the stimulation received from the cropsac was inadequate to support regurgitation activity during parental feeding interactions with the squab. Collectively, these results suggest that prolactin acts at both central (i.e.

brain) and peripheral (i.e. crop) sites to promote this behavior. The relative importance of these two modes of prolactin action remains to be established.

The integrity of the preoptic area (POA) of the dove brain appears to be crucial to the expression of prolactin-induced parental regurgitation feeding activity. Axon-sparing lesions of the POA caused profound deficits in parental regurgitation behavior that were induced by systemic injections of prolactin (116). Importantly, crops were well developed in these prolactin-treated, POA-lesioned birds, thereby ruling out a possible indirect effect of the lesion on parental activity. The observation that the prolactin-induced orexigenic response (stimulation of appetite) was intact in POA-lesioned animals is also significant because it suggests that the deficits observed were specific to parental behavioral responses.

Prolactin probably plays a role in inducing a generalized behavioral state of enhanced parental responsiveness, of which parental regurgitation feeding behavior is only one component. Prolactin has been reported to increase the incidence of defensive and aggressive behavior when administered systemically to nonbreeding doves (123). Prolactin also enhances the defensive responses elicited by electrical stimulation of the paleostriatum (74). These responses parallel the increase in nest defense that typically occurs during incubation and the early posthatching period of the breeding cycle (74, 77, 79). Moreover, prolactin increases the preference of male ring doves for squab in a squab vs egg choice test (30). This finding reinforces the view that, at least in this species, prolactin may preferentially support behavioral adaptations associated with interactions with young.

Is Prolactin Essential for Parental Responsiveness?

The above evidence clearly suggests that prolactin facilitates the display of parental regurgitation feeding behavior and associated activities in columbids. However, whether this hormone is essential for the expression of these behaviors has yet to be determined conclusively. Passive immunization against vasoactive intestinal polypeptide (VIP), a neuropeptide with potent prolactin-releasing effects in doves and other birds (78, 111), did not disrupt incubation behavior or parental feeding of young in ring doves even though the treatment significantly depressed plasma prolactin concentration and almost completely prevented cropsac development (76). Although these findings have raised questions concerning prolactin's role in incubation behavior and its effect on parental responses toward young, other interpretations cannot be ruled out. Total suppression of the incubation-associated rise in plasma prolactin was not achieved by the VIP antiserum, and the suppressive effects themselves were short-lived because of immunoneutralization of the exogeneously administered antibodies. Accordingly, it is possible that the transient decrements in prolactin secretion were not sufficient to disrupt ongoing behavior, or that sufficient

prolactin was secreted to support parental behavior changes even though levels were not adequate to promote cropsac growth.

Some investigators (115) have cited the observation that ring doves will exhibit parental regurgitation toward foster young during early incubation, when plasma prolactin and crop development are still at basal levels, as additional evidence against an obligatory role of prolactin in parental behavior (55, 56, 71). However, the interpretation of this observation is complicated by the fact that the squab exposure itself induces prolactin release in ring doves tested during early incubation, as measured by accelerated cropsac growth (55, 56). Whatever role prolactin may play in the initiation of parental regurgitation behavior, its influence may wane during the latter stages of the posthatching period because parental feeding of young continues during the late posthatching period after plasma prolactin and cropsac development have returned to basal levels. This interpretation is consistent with a general pattern seen in a variety of vertebrate species in which parental responsiveness becomes progressively less dependent on hormonal influences and progressively more dependent on stimuli from the young as the parental phase of the reproductive cycle proceeds (103).

When evaluating the issue of prolactin involvement in parental responsiveness, one must consider the stimulus context in which these behaviors are exhibited. Laboratory studies are typically conducted under optimal conditions in which the birds are confined to small breeding cages with continuous exposure to the nest and unlimited access to food and water. Accordingly, as Lea et al (76) have suggested, the role of prolactin in parental behavioral expression may well be more important under the suboptimal conditions normally encountered by wild populations, when food availability and time spent in close spatial proximity to the nest may be greatly reduced. An accurate and definitive characterization of the role of prolactin in parental behavior may therefore require additional studies of birds living under both laboratory and field conditions.

PROLACTIN AND PARENTAL HYPERPHAGIA

In addition to ensuring an adequate supply of cropmilk for the newly hatched squab, prolactin also may stimulate feeding activity by the parents to supply seed and other foodstuffs as the nestlings grow older. Patterns of feeding behavior are generally similar in the pigeons and doves that have been studied. In ring doves (*Streptopelia risoria*), food consumption by the breeding pair remains relatively constant from the prelaying period through most (8, 75) of the incubation phase, whereas in domestic pigeons (*Columba livia*), food intake is diminished during most of incubation (92). Despite these differences, both species exhibit a marked and sustained increase in food consumption during

the posthatching period in a pattern that parallels the nutritional demands of the squab (Figure 1). Young squab are fed almost exclusively on cropmilk produced by both parents during the first few days of life (95, 122), and daily food intake of the parents does not exceed prelaying levels during this time (75, 92). Nevertheless, short-term changes in parental food consumption at the time of hatching may be more dynamic. Lea et al (75) reported a significant decline in food intake in breeding ring dove pairs during the last four days of incubation, followed by a prompt and significant rebound in feeding within 24 h of hatching. Food intake continued to increase on subsequent days. That posthatching hyperphagia is not accompanied by an increase in body mass is significant. In fact, body weight declines significantly during this period, more quickly in parents feeding two squab than in those feeding only one (92, 120). This observation indicates that the additional food consumed after hatching is not used by the parents but is instead transferred to the young (92).

Prolactin Involvement in Parental Hyperphagia

Plasma prolactin is elevated during the posthatching period, when parental food consumption is increased to meet the nutritional demands of the growing squab (Figure 1). This finding, together with evidence that prolactin stimulates feeding behavior in pigeons and doves, suggests that prolactin may promote parental hyperphagia in columbids. Schooley et al (108) were the first to demonstrate that prolactin is effective in countering the decline in food intake and body weight that occurs following hypophysectomy of pigeons. Bates et al (3) reported a significant orexigenic effect of a highly purified bovine prolactin preparation. However, the elevation in food intake induced by prolactin alone was less than that induced by the combination of prolactin, thyroxine, and prednisone. Therefore, the prolactin-induced hyperphagia (108) depends on the permissive action of other hormones.

Ovine prolactin treatment results in a doubling of daily food consumption and a 20% gain in body weight in intact photostimulated doves (14) when given either systemically or by ICV injection (14, 19). Prolactin also stimulates water intake. However, it is unlikely that prolactin stimulates drinking directly since it elevates feeding in water-deprived doves but has no effect on drinking in food-deprived birds (10).

Prolactin-induced hyperphagia is sexual dimorphic. In male doves, prolactin stimulates doubling of daily food consumption and results in a 20% increase in body weight over a 10-day period. This increase is approximately twice that observed in females treated identically (14, 15). This marked sexual dimorphism may be an adaptation to the columbid breeding strategy, wherein the interval between successive clutches varies in response to changes in food availability (20, 92). Under favorable conditions, the female lays a new clutch of eggs while the young from the first clutch are still dependent on the parents

for food. With the female occupied with the new clutch, the male assumes the principal duties of feeding the nestlings (20, 92). The more pronounced prolactin-induced feeding response of the male is therefore consistent with the male's greater parental investment at this stage. At present, the physiological basis of the sex difference in feeding response is unknown. Because androgen promotes feeding activity in castrated pigeons (99), it is conceivable that differences in gonadal steroids are responsible for the sex-specific patterns in behavioral response to prolactin. However, this hypothesis is difficult to evaluate because ICV injections of prolactin result in pronounced gonadal regression and suppression of gonadotropin secretion in both sexes (11, 16, 19). Whatever the case, a reduction in the secretion of testicular androgen is not a prerequisite for prolactin-induced hyperphagia, as pronounced prolactin-induced feeding responses are observed in castrated birds given sufficient testosterone to restore precastration levels of androgen-dependent courtship behavior (J Richards & JD Buntin, unpublished results). This finding indicates that prolactin's hyperphagic effects are not secondary to its antigonadal action.

Although a detailed discussion of the role of prolactin in the regulation of food intake in other species of birds is beyond the scope of this review, it is worth noting that the prolactin-induced hyperphagia that occurs in columbid species is not observed in all birds. For example, although prolactin strongly stimulates feeding activity in ducks (40) and has been linked to premigratory hyperphagia and fattening in songbirds (90), it apparently contributes to the anorexia seen in turkey hens during incubation (29, 130). In some subtropical passerine species, prolactin reportedly causes changes in body weight by altering the daily periodicity of feeding and efficiency of food utilization in addition to altering the amount of food consumed (22, 27). These effects illustrate the diversity of prolactin action in regulating feeding and underscore the complex role of prolactin in promoting the highly specific adaptations in nutrient partitioning that are seen across different avian taxa.

CNS Mediation Of Prolactin-Induced Hyperphagia

A central site of prolactin action is suggested by the robust hyperphagia in ring doves given intracranial injections of prolactin at doses below those necessary to stimulate peripheral target tissues. Nevertheless, the neural events that mediate the orexigenic effects of prolactin are poorly understood. In male doves, an increase is observed in total time spent feeding and average feeding-bout duration following a single ICV injection of prolactin (10). The response is slow to develop, and consistent differences in the amount of seed consumed by prolactin-treated males and vehicle-treated controls are not detectable until 6–10 h after injection. This latency in the feeding response is considerably longer than that observed following ICV administration in other species of several other orexigenic peptides, such as neuropeptide Y (25, 73),

galanin (105), growth hormone–releasing hormone (121), and opioid peptides (31, 93). It also exceeds the response latencies reported for other prolactin-induced behavioral changes following intracranial injection (29, 34, 35). Another remarkable feature of this response is its protracted duration, with significant elevations in feeding still detectable 24–48 h after a single ICV injection of the hormone (10). The delayed onset and extended time course of the response presumably reflect the complexity of the intervening molecular, neurochemical, and neurophysiological events underlying these prolactin-induced changes.

When ovine prolactin was administered once daily by ICV injection, the magnitude of the hyperphagic response increased directly with ovine prolactin dosage at concentrations above the threshold dose of 0.1–0.5 μg (4–22 pmol)/day (14). At the highest dose of prolactin tested (2 μg/day), average daily food intake was 90% higher than that recorded during the pretreatment period, whereas at 1 μg/day, a 60% increase was observed. Paradoxically, turkey prolactin was ineffective in elevating food consumption at the 1 μg/day dosage (14). Similar potency differences are observed when prolactin-induced changes in plasma LH and testes weight are measured (16) and when prolactin preparations from the two species are compared as competitors of ^{125}I-ovine prolactin for binding to receptors in dove liver (15) and brain (17). Binding studies with pigeon or dove prolactin preparations are needed to clarify these relationships.

Interestingly, growth hormone preparations are approximately equivalent to prolactin in orexigenic potency when administered ICV at 1 μg/day (14). These results are consistent with earlier work demonstrating orexigenic effects of systemically administered mammalian growth hormone preparations in hypophysectomized pigeons (3). Although the efficacy of human growth hormone could perhaps be attributed to its documented ability to interact with prolactin receptors in dove brain (17) and other tissues (97), this line of reasoning cannot explain the orexigenic properties of turkey and ovine growth hormone, which bind to prolactin receptors in dove brain with low affinity (17, 18). These data, together with recent evidence for the presence of growth hormone receptors and receptor mRNA in chicken brain (1, 45), suggest that both prolactin and growth hormone are orexigenic in columbids. To date, however, attempts to detect growth hormone binding sites in dove brain have been unsuccessful (RM Hnasko & JD Buntin, unpublished results).

Neural Sites Mediating Prolactin-Induced Feeding Activity

Recent evidence strongly supports the hypothesis that prolactin interacts with specific populations of prolactin receptors in the dove brain to promote hyperphagia. Anatomically discrete, saturable populations of prolactin binding sites have been detected in dove brain by in vitro competitive binding studies and quantitative autoradiography using ^{125}I-ovine prolactin (17, 18, 41). These re-

ceptors share the high binding affinity ($k_d \approx 10^{-10}$M) and hormonal specificity of their counterparts in peripheral target tissues and appear to be accessible to blood-borne hormone (17, 18). Prolactin receptors are nonuniformly distributed in dove brain and tend to be mainly concentrated in the preoptic area and in several regions of the hypothalamus. Several of these prolactin-sensitive regions, most notably the paraventricular nucleus, the ventromedial nucleus, and the lateral hypothalamic area, are also involved in regulation of food intake in birds and mammals (6, 69, 72, 83, 84, 117). Microinjection of ovine prolactin to a variety of prolactin-sensitive neural sites implicates three brain regions as mediators of the prolactin-induced feeding response in male ring doves: the preoptic region, the ventromedial hypothalamic nucleus (VMN), and the tuberal region of the hypothalamus (42, 58). Although prolactin enhanced feeding activity when injected into each of these three sites, the increase in food intake induced by prolactin injected at the VMN was significantly greater than that induced by microinjection at any of the other sites tested (58). These results imply that the VMN is the most important site of prolactin action in promoting the feeding response. Antibodies to the rat liver prolactin receptor inhibit the specific binding of ^{125}I-ovine prolactin to the dove VMN and markedly diminish the hyperphagia caused by VMN injection of prolactin (87).

Of potential significance in understanding prolactin-induced alterations in feeding behavior is accumulating evidence for prolactin-like molecules of brain origin. Immunocytochemical or biochemical evidence for brain prolactin is available from one avian species (5) and from a variety of other vertebrates (32, 33, 39, 47, 54, 104, 127). To date, prolactin-like immunoreactivity in ring dove brain has not been detected using a heterologous antibody (RM Hnasko & JD Buntin, unpublished results).

Although several hormones have been implicated as potential modulators of prolactin-induced feeding responses, the precise nature of the hormonal interactions involved has not been adequately characterized. Gonadal steroids may contribute to the sex differences observed in prolactin-induced feeding responses in ring doves, but for reasons described above, they are unlikely to mediate the prolactin-induced feeding response itself. As discussed above, evidence from studies involving central administration of prolactin and growth hormone suggests that these two hormones act independently to promote feeding behavior in ring doves. On the other hand, systemic injection studies reveal a complex pattern of hormonal interactions. In tests with hypophysectomized pigeons, both prolactin and growth hormone were orexigenic when injected alone but exhibited no additivity or synergism when administered in combination (3). In contrast, additive effects were observed when these hormones were coadministered with thyroxine and prednisone. Additional studies are clearly needed to clarify these hormonal relationships.

Systemic injection studies in hypophysectomized pigeons indicate that ad-

renal corticosteroids amplify the effects of prolactin on feeding activity (3, 91). However, these corticosteroids do not seem to have significant orexigenic properties of their own. This lack of a significant feeding response to adrenal corticosteroid administration is consistent with results obtained in dark-eyed juncos (*Junco hyemalis*) (52) but stands in contrast to the hyperphagia reported by Nagra et al (96) in pheasants (*Phasianus colchicus*) and by Bartov et al (2) in chickens. In pigeons, the relationship between corticosterone and prolactin may be complex, as the magnitude of prolactin-induced fattening and growth of cropsac is strongly influenced by changes in the phase relationship between the daily rhythm of corticosterone and the timing of prolactin injections (see 89 for review). Interestingly, plasma corticosterone concentrations increase significantly after hatching in the ring dove (75). Assuming similarities in the hormonal control of food intake in pigeons and doves, this increase raises the intriguing possibility that parental hyperphagia results from synergistic interactions between corticosterone and prolactin during the posthatching period.

In contrast to adrenal corticosteroids, thyroid hormones did not synergize with prolactin in promoting food intake when administered to hypophysectomized pigeons in the absence of other hormones (3, 91), nor did they augment the feeding responses induced by the combined administration of desoxycorticosterone acetate and prolactin in these birds (91). In addition, thyroid hormone concentrations in plasma are low during the brooding period of the ring dove breeding cycle, when elevated prolactin and hyperphagia are observed (75). These data provide evidence against a significant role of thyroid hormones in prolactin-modulated changes in food intake.

CONCLUSIONS

The coordinated regulation of cropsac development and the physiological and behavioral support of milk feeding in pigeons and doves is highly similar to the role of prolactin in mammals. All mammals commit large amounts of parental resource to the success of a limited number of offspring. These commitments are made throughout the gestational and postgestational feeding periods. Prolactin plays an important role in the biology of mammalian parenting, from support of luteal function in some species to regulation of lactogenesis in others. Likewise, in pigeons and doves, prolactin figures prominently in the regulation of parental commitment. Other vertebrate examples of prolactin involvement in parental functions are well known (59).

Although the role of prolactin in parenting is common among vertebrates, it is not universal, and the physiological and developmental basis of its involvement are entirely different in the various groups. The cropsac and mammary gland have little in common at the physiological level other than their dependence on prolactin. Therefore, the explanation for the convergent roles

of prolactin in parenting must lie outside the particulars of the physiological processes used in the various vertebrate groups. Theoretically, the antigonadotropic activity of prolactin, which is conserved throughout the vertebrates (59), ensures that prolactin secretion is highest during the postmating period of the breeding cycle, when parental functions can be stimulated. The evolutionary choice to invest parental resources in feeding the offspring has been made independently in several groups of vertebrates. Postmating secretion of prolactin appears to be a necessary prerequisite for adoption of that strategy.

At a molecular level, the mechanisms of prolactin actions are highly conserved. The pigeon prolactin receptor is functional in both binding and signaling assays in mammalian cells, and the protein kinases and transcription factors used in birds and mammals appear to be similar. Continuing studies on the mechanisms of prolactin actions at the molecular and physiological levels will elucidate not only the details of the conserved mechanisms of this hormone, but also the specific adaptations that allow for specialized functions, such as feeding of cropmilk and mammary-gland milk.

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