Parental Care and the Prolactin Secretion Pattern in the King Penguin: An Endogenously Timed Mechanism?

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The King penguin (*Aptenodytes patagonicus*) has been studied on Possession Island, Crozet Archipelago (46°25'S-51°45'E). It is an offshore feeder, but it breeds on land. Its breeding cycle is unusually long (about 14 months). It starts at the beginning of spring, is interrupted during 5 months of winter, and ends in the next spring. Furthermore, it is characterized by a long parental care period, of about 11 months, including the winter interruption. In fact, care given to the egg and the chick is biparental, which supposes that parental behavior includes both parents. Each parent alternates care given to the egg and to the chick on land and foraging bouts at sea. An incubation bout, or a chick care bout, is called a shift. Prolactin is the hypophyseal hormone known to be correlated with incubation and chick care. We studied the mechanism of the maintenance of prolactin during the parental care period in the King penguin, a period which is unusually long. In many species, prolactin secretion has been shown to be stimulated by the presence of eggs and/or chicks, but in the King penguin, prolactin secretion is observed throughout the entire period of parental care, despite the fact that the birds leave the egg and the chick repeatedly and for extended periods of time to feed. Prolactin levels rise significantly at the beginning of courtship; females have significantly higher prolactin levels than males during courtship, copulation, and the period of waiting for egg laying. In both sexes, prolactin levels remain high during incubation and the first part of chick rearing, before winter. Prolactin concentrations decline somewhat during the winter period of minimal parental care, but remain that level in spring when parental care starts again. The level returns to basal value during molt. Prolactin levels rise during the incubation shifts but not over the course of contact with young. Prolactin values remain high in unsuccessful breeders, possibly preventing the birds from relaying, but remain low in immature birds. These data raise questions about how prolactin secretion is controlled in this species. The hypothesis of a programmed secretion of prolactin is advanced. © 1996 Academic Press, Inc.

For many species, especially among mammals and birds, a reproductive effort is prolonged by parental behavior. In its broadest sense, parental behavior includes the preparation of nests or burrows, the production of the eggs, incubation, and the rearing of the young. This latter period is absent or very limited in precocial species (Rosenblatt, 1992), but in altricial ones, it is more or less developed. In its more extended form, it includes the brood stage (the chick, not thermally emancipated, is still incubated), chick care (when the chick can be left alone in the nest or in creche with others), and postfledging care (despite the chick's ability to fly or swim) (Burger, 1980).

Since Riddle *et al.* (1935), an hypophyseal hormone, prolactin, has been known to play an important role in parental behavior. In numerous cases, this hormone was said to be correlated with parental care, rising just before incubation and dropping after hatching, in precocial species (duck: Hall and Goldsmith, 1983; hen: Zadworny *et al.,* 1988) or dropping at the end of parental care (Rosenblatt, 1992) in altricial species. However, patterns of prolactin secretion do not seem to be so strict or general (Open and Proudman, 1980; Phillips *et al.,* 1985). For some species, incubation starts although the prolactin level is not elevated (canary: Ball, 1991; Goldsmith, 1982; ring dove: Goldsmith *et al.,* 1981), and the level of this hormone may drop although parents still take care of their chick (pied flycatcher: Goldsmith, 1983; Harris' Hawk: Masters Vleck *et al.,* 1991).

For many species, prolactin secretion is initiated and

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maintained by nervous stimulation, visual, aural, or tactile, and generally drops in the absence of this stimulation. For instance, tactile stimuli, from eggs and the nest itself, are implicated in prolactin secretion and maintenance of this secretion in hens (Richard-Yris *et al.,* 1987; Sharp *et al.,* 1988; El Halawani *et al.,* 1990) and in ring dove (Goldsmith, 1983).

King penguins (*Aptenodytes patagonicus*), living in subantarctic regions, are subjected to severe environmental factors. Their breeding cycle is adapted to those ecological constraints. The adult birds arrive at the nest site from mid September to January. After the courtship period, lasting ca. 3 weeks, laying (one egg) is followed by incubation. In birds, this behavior is biparental in over 90% of all species and in 100% of seabirds (Lack, 1968). In King penguin, the mean duration of incubation is 55 days. The first incubation bout, also called a shift, is carried out by the male, as the female goes to sea a few hours after having laid the egg. Incubation shifts last about 17 days. The hatching period occurs from January to April. The chick is brooded for about 32 days after hatching, with shifts lasting about 6 days. Then the chick joins other chicks in a crèche for about 11 months, including the winter period. During winter, chicks are scarcely fed. Feeding starts again in September for at least 2 months, until chicks molt and depart at the end of November onward (Weimerskirch *et al.,* 1992).

Thus, the parental care period is unusually long. Furthermore, in response to ecological constraints, mainly exploitation of pelagic resources several hundred kilometers from the breeding area, the King penguin is an offshore feeder (Jouventin *et al.,* 1994), and incubation shifts are themselves long (about 17 days). Moreover, chick rearing is interrupted during approximately 5 winter months (Cherel *et al.,* 1987). During this period, food supply is short and localized near the polar front, to more than 3000 km from the breeding area (Jouventin *et al.,* 1994). The energetic effort necessary to come back to the land is considerable. Thus, during winter, only 26% of chicks were fed once and 26% were fed twice, so that the chicks presented a 68% decrease in body mass (Weimerskirch *et al.,* 1992). These unusual characteristics led us to investigate the endocrine mechanisms underlying the maintenance of parental behavior in this species, despite the absence most of the time of the stimuli that have been shown to elicit prolactin secretion in most species.

METHODS

Field Procedures

The study was carried out on Possession Island, Crozet Archipelago (46°25′S-51°45′E). The King penguin

TABLE 1

Abbreviations, Stages of the Breeding Cycle, and Mean Duration of the Stages

Abbreviations	Stages	Mean duration
MO	Molt	About 3 weeks
EM	The end of the molt	
CO	Courtship	8 to 15 days
COP	Copulation	
WL	Waiting for egg laying	About 1 week
INC	Incubation	54 to 56 days
CRI	Chick rearing before winter	About 3 months
WCR	Winter chick rearing	About 4 months
CRII	Chick rearing after winter	About 2 months

breeding colony, situated at the Crique de la Chaloupe, had about 700 breeding pairs of which half were identified with banding.

King penguins were sampled at different behavioral stages of their breeding cycle (Table 1) determined by Jouventin (1978).

Additional blood sampling was done in birds that failed to breed, i.e., unsuccessful breeders, and in immature animals.

Five milliliters of blood was collected from the brachial vein of the flipper into heparinized tubes. Samples were centrifuged at 4°C for 10 min at 3000*g,* immediately after collection; the plasma was decanted and stored at −25°C until it was assayed in France. The time of day at which the samples were drawn was recorded. However, we found no trends in plasma levels of prolactin as a function of time of sampling.

Prolactin Radioimmunoassay

The radioimmunoassay of blood samples was conducted according to the method previously described and validated for King penguin plasma in our laboratory (Cherel *et al.,* 1994). All plasma samples were assayed at the same time in duplicate to eliminate interassay variation. The within assay coefficient of variation for prolactin was 3%.

Statistical Analysis

Data were analyzed statistically by analysis of variance (after testing homogeneity of intragroup variances) and by the Newman–Keuls multiple range test. Throughout the text, mean hormone concentrations are given ± SE.
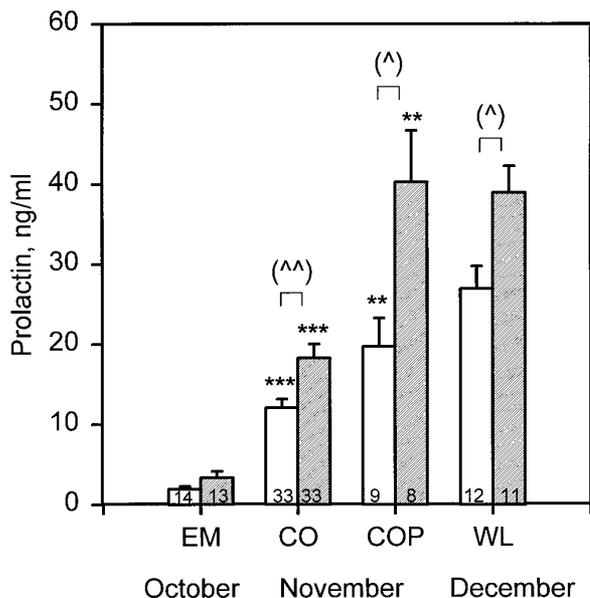
FIG. 1. Changes in plasma prolactin concentrations in male (open bars) and female (hatched bars) King penguins, from the end of the molt to the period of waiting for egg laying. Values are means (ng/ml) ± SE. Sample sizes are given inside of the bars; asterisks indicate significant differences from the previous data point; and carats indicate significant differences between male and female: $**, \wedge P < 0.05$, $***, \wedge \wedge P < 0.01$. Abbreviations of the breeding stages given at the bottom of the figure (see also Methods) are EM, end of the molting period; CO, courting period; COP, copulation; WL, period of waiting for egg laying.

RESULTS

Prolactin Concentration at the Beginning of the Breeding Cycle (Fig. 1)

In both sexes, plasma prolactin concentration was at a basal level at the end of the molt (EM). It rose significantly during courtship (CO) until copulation and remained at that high level during the period of waiting for egg laying (WL). Prolactin concentrations were significantly higher in females than in males during courtship ($P < 0.01$), copulation ($P < 0.05$), and the period of waiting for egg laying ($P < 0.05$), but not significantly at the end of the molt.

Prolactin Concentration during the Entire Parental Care Period (Fig. 2)

From the incubation period onward, there was no significant difference between male and female prolactin concentrations. Thus, mean values presented in Fig. 2 are pooled data. Plasma prolactin concentration

peaked during incubation period (INC) and remained significantly elevated during the entire parental care period compared to the basal value at molt (MO) (molt compared to chick rearing I, $P < 0.01$). However, considering the different stages of the chick rearing period, we noticed that the plasma concentration during the chick rearing I (CRI), before the winter interruption, was significantly higher than the ones observed during winter (WCR, $P < 0.01$) and after winter (CRII), with no significant difference between the latter sampling points.

Prolactin Concentration during an Incubation Shift (Fig. 3)

In birds arriving at the colony after a period of feeding at sea, before taking the egg from the partner to incubate it, prolactin concentration is high (44.34 ± 1.87 ng/ml). During a mean incubation shift of 18 days, three periods were considered in the variation of prolactin concentration. For the first period, from egg exchange to the 6th day of incubation, prolactin values
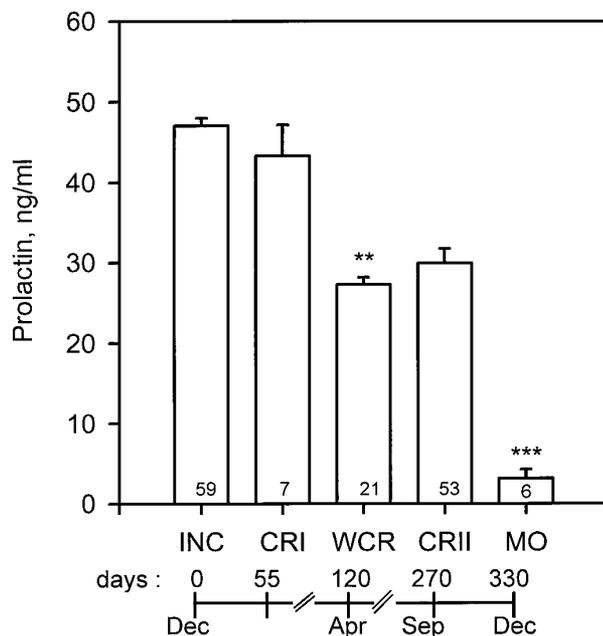

FIG. 2. Changes in plasma prolactin concentrations in King penguins, from the incubation period to the molt (prolactin concentrations of males and females were pooled). Values are means (ng/ml) ± SE. Sample sizes are given inside of the bars; asterisks indicate significant differences from the previous data point: $**P < 0.01$; $***P < 0.001$. Abbreviations used: INC, incubation; CRI, first period of chick rearing; WCR, winter chick rearing; CRII, second period of chick rearing; MO, molt.
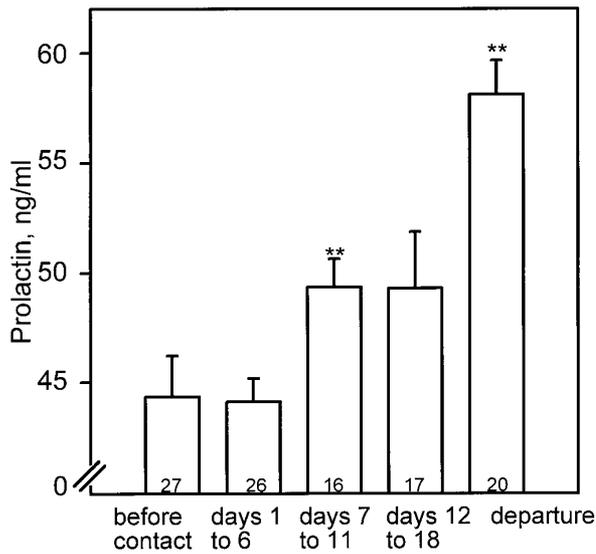
FIG. 3. Changes in plasma prolactin concentrations during an incubation shift (prolactin concentrations of males and females were pooled). Values are means (ng/ml) ± SE. Sample sizes are given inside of the bars; asterisks indicate significant differences from the previous data point: **$P < 0.01$.

FIG. 4. Prolactin concentration during chick feeding bouts, before and after feeding and during and after winter. Values are means (ng/ml) ± SE. Sample sizes are given inside of the bars.

remained stable. Prolactin concentration significantly rose from the 7th day ($P < 0.01$). It tended to rise further, reaching a higher level at the end of the incubation shift when the mate came back from the sea.

Prolactin Concentration before and after Chick Feeding (Fig. 4)

Contrary to observations during incubation, prolactin levels did not fluctuate during a chick feeding bout, during winter or spring rearing periods. There was no significant difference in prolactin levels of birds arriving from the sea to feed their chick and prolactin levels of birds returning to sea after having fed their chick.

Prolactin Concentration in Nonbreeding vs Breeding Birds (Table 2)

In general, the birds which failed their reproductive attempt, whatever the stage, rapidly left the colony and only returned occasionally. Thus sample size was small. For birds which had failed recently (in summer) or earlier (in winter), the prolactin level remained high compared to the basal value at the end of the molting period ($P < 0.01$). Moreover, there is no significant difference between those two values. However, prolactin concentrations of birds which failed were significantly lower
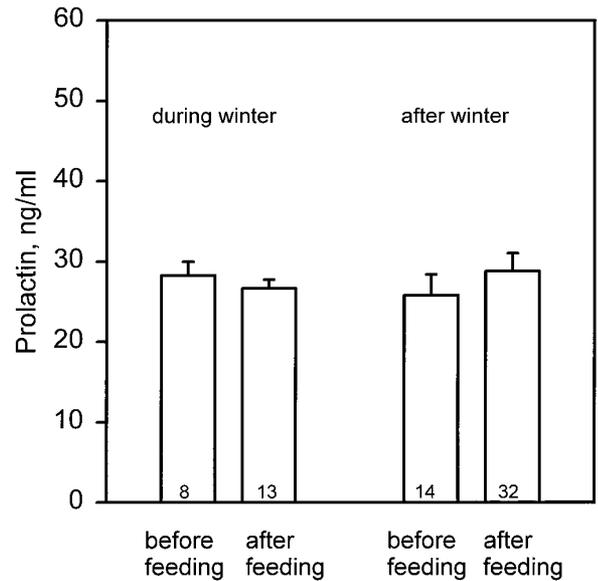
than prolactin concentrations of birds which incubated ($P < 0.001$). Prolactin concentration started to rise at the end of courtship in summer. However, it did not rise in immature birds. In these birds, prolactin levels were very low, even though they were significantly higher than in birds at the end of the molt ($P < 0.01$).

DISCUSSION

Initiation of Prolactin Secretion

At the end of courtship, in December to January, prolactin starts to rise in breeders, reaching a peak during the incubation period. As for many species, prolactin is associated with parental behavior (Rosenblatt, 1992),

TABLE 2

Prolactin Concentrations in Nonbreeding vs Breeding Birds (Male and Female Prolactin Concentrations Are Pooled)

	Mean (ng/ml)	SE	n	Season
Unsuccessful breeders	26.03	4.93	5	Summer
Unsuccessful breeders	22.42	1.57	8	Winter
Incubating birds	47.02	0.99	59	Summer
Nonbreeders (end of molt)	2.60	2.20	27	Summer
Immature birds	4.73	0.43	21	Summer

but it starts to increase before incubation, as demonstrated in hens (Lea *et al.,* 1981). In King penguins, females had significantly higher prolactin levels than males during the prelaying period. These results are in agreement with those obtained in other biparental penguin species such as the Macaroni and Gentoo penguins, in which prolactin increased earlier in females than in males (Williams and Sharp, 1993). In some bird species, prolactin has been reported to be driven by the photoperiod. In starlings, for example, a seasonal cycle of prolactin secretion occurs independently of reproduction and is observed in nonbreeding adults and in immature birds (Dawson and Goldsmith, 1985). It occurs even in males, although only females incubate (Dawson and Goldsmith, 1985). The results reported by Williams and Sharp (1993) showed an increase in prolactin secretion in immature Macaroni and Gentoo penguins at the same time as the increase in breeders, which could imply the role of photoperiod in these species. In our study, immature King penguins sampled during the period of longest daylength, December–January, exhibited prolactin levels that were at basal values. We did not found any seasonal trend in prolactin from birds courting early, in October, or late, in January, in the breeding season (data not shown). It seems therefore, that in the King penguin, it is not the photoperiod which initiates the prolactin rise in breeders.

Role of Environmental Stimuli in the Maintenance of Prolactin Secretion

In many species, prolactin secretion has been shown to be stimulated or maintained by the presence of eggs and/or chicks (El Halawani *et al.,* 1990). A brood patch, a defeathered and highly vascularized region where the egg or the chick is incubated, is usually formed (Jones, 1971). During incubation, tactile stimuli induce or maintain prolactin secretion. In female ducks, for example, prolactin levels dropped if the brood patch had been anesthetized (Hall and Goldsmith, 1983). In King penguin, stimulation of the brood patch could be responsible for the difference in prolactin levels recorded between the partner incubating and the one returning from the sea. Nontactile cues such as visual or auditory signals from the chicks might also contribute to the maintenance of prolactin secretion during an incubation or feeding shift.

The Prolactin Secretion Might Be Endogenously Programmed

In most species, prolactin declines drastically within one or a few days when incubation is interrupted. In hens deprived of their nests, plasma prolactin returned to baseline values 48 hr after nest deprivation (Sharp *et al.,* 1988). In the Cape gannet, a wild bird species comparable to the King penguin in that it has a single egg and both sexes share equally in incubation duties, prolactin levels decreased significantly within 24 hr after removal of the egg (Hall, 1986). During the 11-month parental period, King penguins leave their eggs and their chicks repeatedly for extended periods of time to feed, especially in winter. So, one wonders how parental behavior is maintained.

It is not possible to obtain information on prolactin levels in birds that are foraging at sea hundreds of kilometers from the breeding area. However, parents arriving to start a shift had elevated prolactin levels before they reached their chicks, not only during the brooding phase but also during the crèche stage from autumn to spring. In an experimental manipulation reported elsewhere (Jouventin and Mauget, 1996), we kept failed breeders in individual enclosures. In these birds, prolactin concentration did not decrease significantly during the 48 hr following egg loss. The results presented here (Table 2) show that, even in unsuccessful breeders, prolactin titers did not drastically drop. Since prolactin does not return to basal levels despite the absence of stimulus, another mechanism may be operating. Prolactin secretion may be endogenously programmed. Such a mechanism may occur in other avian species. It may explain, for example, the strict prolactin elevation in the pied flycatcher, lasting 16 days, which involves the normal incubation period and a few days after hatching (Silverin and Goldsmith, 1984). This period of elevation can be experimentally shortened by 2 to 4 days, but not extended. Experimentations consist of substitutions of old eggs by younger ones and vice versa. Endogenous programming may also explain results of the Wandering albatross. For this bird, prolactin secretion begins in the middle of the incubation period and stops after 68 days. Prolactin secretion in this species can neither be shortened nor extended (Hector and Goldsmith, 1985).

Several alternative hypotheses may account for the control of prolactin secretion. Prolactin could be regulated by metabolic changes. The nutritional status of birds was not investigated in the present study. However, we have previously shown that prolactin levels remained unaffected in King penguins that fasted for several weeks (Cherel *et al.,* 1994). Prolactin decreased only when fat stores reached a critical depletion when birds were in the late phase of fasting (phase III, Cherel *et al.,* 1988). The birds sampled in this study never reached this critical phase.

Another interpretation of the maintenance of prolac-

tin secretion could be that elevated levels of prolactin are a result of reproductive photorefractoriness. In most species exhibiting photorefractoriness, high prolactin is associated with refractoriness (Nicholls *et al.,* 1988). However, it is not clear wether King penguins exhibit true refractoriness. GnRH challenge studies done by Jouventin and Mauget (1996) have shown that the responsiveness of the pituitary gland paralled seasonal changes in photoperiod. This pattern was comparable to that of bird species (e.g., of the genus *Columba*) that do not become photorefractory.

Finally, even if some caution is needed, our data are suggestive of endogenous programming of prolactin secretion in the King penguin. Such a mechanism would be an adaptation associated with long parental care and lengthy periods of absence in birds breeding under harsh environmental conditions. It is reasonable to hypothesize that most offshore seabirds could present this adaptation, but also other species going far from the nest to find food for chicks (vultures, for example) or other long-lived birds with incubation shifts that last several days or weeks. This original mechanism, when we compare with the present literature concerning mainly small passerines, is probably the only one that allows these species to breed under these conditions.

ACKNOWLEDGMENTS

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