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Changes in Prolactin in Peripheral Plasma during Lactation in the Brushtail Possum *Trichosurus vulpecula*

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Abstract

A heterologous double-antibody radioimmunoassay has been validated for prolactin in plasma and pituitary preparations of *T. vulpecula*. Serial dilutions of crude pituitary homogenates and plasmas from several marsupials and purified prolactin from the tammar, *Macropus eugenii*, showed parallel dose response curves. In both male and female possums plasma prolactin concentrations increased in response to a single intravenous injection of thyrotrophin releasing hormone.

Plasma prolactin concentrations were measured in six lactating females (June–November) and in four non-lactating females (July–October). In the following year prolactin levels were also measured in 11 possums with young less than 50 days old and in 24 possums with young aged between 100 and 145 days. In early lactation prolactin concentrations were low (<8 ng/ml) but increased to high levels (>30 ng/ml) by 120 days and remained high until about 160 days of lactation. Thereafter concentrations declined although the young continued to take milk from the mother for a further 30–50 days. The changes in plasma prolactin concentrations throughout lactation are very similar to those described for the tammar, and this unusual pattern appears to be common to marsupials. Non-lactating possums showed no consistent changes in plasma prolactin concentrations between July and October.

Introduction

The common brushtail possum, *Trichosurus vulpecula* (Kerr), (Phalangeridae), an arboreal nocturnal marsupial, is abundant in Australia as well as in New Zealand where it has been introduced. The seasonally breeding female possum is monovular and polyoestrous, the gestation period occupying about 17·5 days of the 26-day oestrous cycle (Pilton and Sharman 1962). Most young are born between April and June, although a second, minor breeding season occurs between September and November. Thus it is possible for a female to raise two young to independence each year (Dunnet 1956; Smith *et al.* 1969). Possum young remain associated with their mother for about 200 days. Between birth and 120 days they are found exclusively in the pouch, while after this time they cling to the mother's back (Dunnet 1956; Smith *et al.* 1969). Weaning is thought to commence at about 130 days and lactation has ceased in most females by 230 days (Smith *et al.* 1969). The adult possum is primarily a folivore and feeds on a wide range of natural and introduced tree species but it may also eat blossom and fruit, as well as grasses (Hume 1982).

During lactation there are marked changes in milk composition (Gross and Bolliger 1959), as well as a large increase in the weight of the sucked mammary gland after 80 days (Smith *et al.* 1969). Similar changes in milk composition, mammary gland weight and development of the young occur during lactation in the herbivorous tammar, *Macropus eugenii* (Macropodidae) (Green *et al.* 1980, 1983; Tyndale-Biscoe

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et al. 1984). Furthermore, in the tammar, there are marked changes in plasma prolactin concentrations during lactation with highest levels occurring, not in early lactation as in eutherians (Cowie *et al.* 1980), but later in lactation coincident with the increase in mammary gland weight, change-over in milk composition and achievement of homeostasis by the young (Hinds and Tyndale-Biscoe 1982, 1985). Therefore it was of considerable interest to determine whether the unusual pattern of plasma prolactin seen in the lactating tammar is a normal feature of lactation common to marsupials. The possum, which is primarily an arboreal folivore, was a suitable species in which to examine this initially since it is a representative of a different family and displays a different life history strategy from the tammar, which is a terrestrial grazer.

Materials and Methods

Animals

Possums (1·8–2·8 kg) were obtained either from the grounds of suburban homes in Canberra or from nearby farming properties. They were captured using cage traps baited with apple and/or aniseed solution and housed in large pens (5 by 3 by 3 m) which contained nest boxes and suspended sacks for shelter. Their diet comprised fresh fruit and vegetables, bread and various types of eucalypt leaves. Water was available *ad libitum*. At capture the young of five lactating females were weighed and measurements of head and pes length taken. Using the growth curves of Lyne and Verhagen (1957) the ages of these young were estimated to be 46, 72, 90, 117 and 129 days. The actual date of birth of a sixth young born in captivity was known. In the following year the stage of lactation of another 35 possums being used in a different study was determined similarly. Eleven of these possums had young aged between 22 and 50 days, while 24 carried young aged between 100 and 145 days.

Collection of Blood Samples

To determine whether the assay would measure changes in prolactin levels *in vivo*, heparinized blood samples (0·8 ml) were collected at frequent intervals via jugular catheters, inserted as described by Khin Aye Than and McDonald (1973), from two intact male and four intact female possums from 60 min before until 200 min after intravenous injection of thyrotrophin releasing hormone (TRH, Roche Australia, 20 μg). Heparinized samples (0·8 ml) were also collected twice weekly from a lateral tail vein of six lactating females from the time of capture until weaning. Four non-lactating possums were sampled twice weekly from July to October. Two of these animals had not produced a young between May and June while the breeding history of the remaining two females was unknown. In the following year 35 lactating possums with young of various ages were sampled once at the time of capture.

After collection blood samples were stored on ice and separated by centrifugation within 30 min. Plasmas were held at -20°C until assayed for prolactin.

Prolactin Assay

Assays for prolactin in possum plasma were carried out by a double-antibody procedure, as described previously for the tammar by Hinds and Tyndale-Biscoe (1982), using antiserum 33/1-8 raised in guinea pigs against human prolactin, and ovine prolactin (NIH-P-S12) as the standard. Duplicate samples of 100 μl plasma were assayed. The accuracy of the assay was determined by the measurement of known amounts of ovine prolactin standard (NIH-P-S12) added to possum plasma containing endogenous prolactin. The precision of the assay expressed as the intra- and interassay coefficients of variation was assessed by the repeated assay of two pools of possum plasma both within and between assays. To assess the specificity of the assay for marsupial prolactin, various pituitary homogenates and purified pituitary fractions from different species of marsupial were assayed. Adenohypophyses were collected within 15 min of death from *T. vulpecula*, *M. eugenii*, eastern quoll (*Dasyurus viverrinus*) and kowari (*Dasyuroides byrnei*), stored at -20°C and crude aqueous homogenates prepared prior to assay. Each anterior pituitary was homogenized in 1 ml assay buffer, serial dilutions (1 : 1000 to 1 : 512 000) prepared and duplicates of 100 μl of each dilution tested in the assay. Dilutions of a purified tammar prolactin fraction [74·9(3)Fr.3] prepared by Dr J. Hawkins using the ammonium sulfate fractionation method of Neill and Reichert (1971) were also assayed. To demonstrate parallelism of immunoreactivity between standard ovine prolactin and endogenous prolactin in plasma, serial dilutions (10–200 μl) of plasmas from four possums and two tammars were tested.

Results

Specificity of the Heterologous Assay for Possum and Tammar Prolactin

Tracer ^{125}I-labelled ovine prolactin bound to antiserum 33/1-8 was displaced in a parallel manner by dilutions of crude pituitary homogenates of all species examined (possum, tammar, eastern quoll, kowari) and by purified tammar prolactin [74·9(3)Fr.3] (Fig. 1). Parallel dose response curves were obtained for serial dilutions

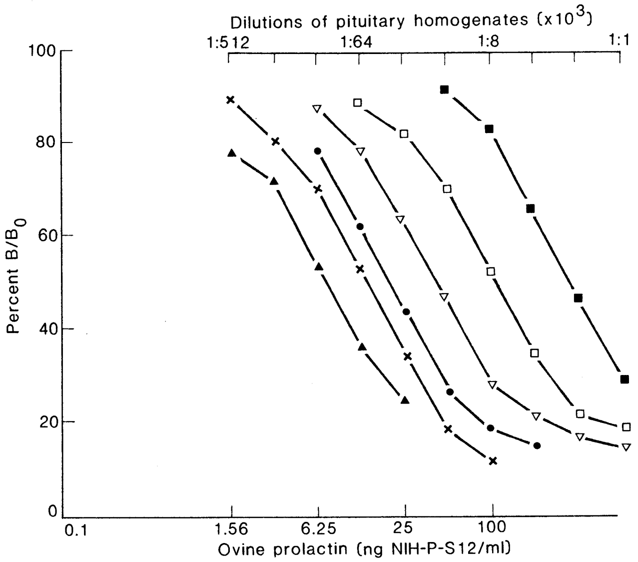

Fig. 1. Inhibition curves for prolactin (semi-log plots, percentage B/B_0) of dilutions of aqueous homogenates of anterior pituitaries of tammar (▲), brushtail possum (▽), eastern quoll (■) and kowari (□); of purified wallaby prolactin [74·9(3)Fr.3] (●); and of ovine prolactin (NIH-P-S12) (×) with antiserum 33/1-8 (1 : 36 000).

of plasmas from an adult male, non-lactating female and lactating female possum and a lactating tammar. No significant displacement of the tracer from the antiserum was induced by plasma from an hypophysectomized female of either species (Fig. 2). Under routine conditions with the antibody dilution fixed at 1 : 36 000 (final dilution) the assay was sensitive to 2 ng/ml plasma, being equivalent to the first significant displacement from zero.

The recovery of ovine prolactin added to a normal possum plasma in which prolactin was not detectable was closely correlated with the amount added ($y = 0{\cdot}969x - 0{\cdot}017$, $r = 0{\cdot}996$, $P < 0{\cdot}001$; $n = 24$) and intra-assay coefficients of variation determined from replicate assays from two pools of possum plasma containing 8·5 and 32·5 ng/ml were 10·0 and 6·5% respectively ($n = 10$ for each pool). Interassay coefficients of variation for the same two pools were 13·0 and 11·0% respectively ($n = 6$ for each pool).

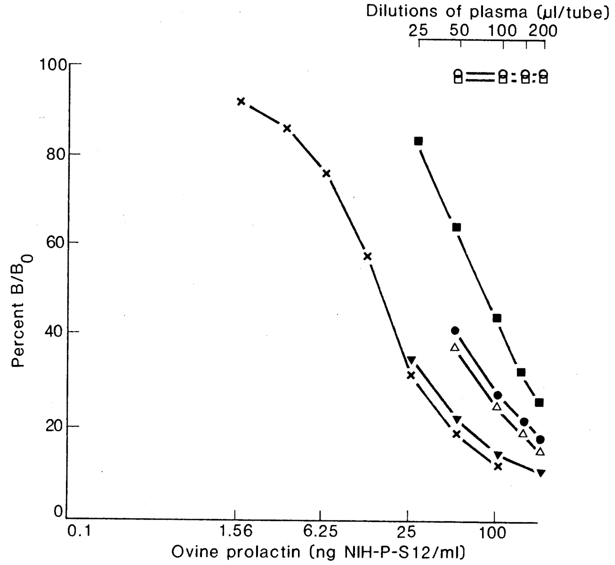

Fig. 2. Dose response curves (percentage B/B_0, semi-log plots) for dilutions of possum plasma in the prolactin radioimmunoassay, using antiserum 33/1-8 (1 : 36 000). Increasing volumes (25–200 μl) of plasma from an adult male (■), a lactating female (●), a non-lactating female (△) and a hypophysectomized female (○) were tested and compared with the standard curve for ovine prolactin (NIH-P-S12) (×). Dilutions of plasma from a lactating tammar (▼) and a hypophysectomized tammar (□) were also tested.

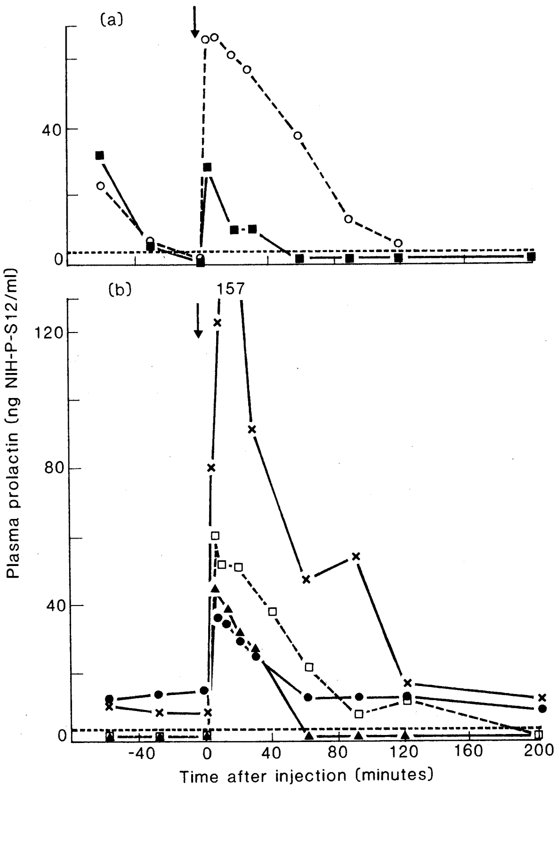

Fig. 3. Effect of intravenous injection of thyrotrophin releasing hormone (20 μg) on plasma prolactin concentrations in (*a*) two adult male possums (○, ■) and (*b*) four adult female possums (×, ●, □, ▲). Solid arrow indicates time of injection. Dashed horizontal line = sensitivity of assay.

Measurement of Plasma Prolactin in Possums: Response to Intravenous Injection of TRH

All of the possums showed an increase in plasma prolactin after an intravenous injection of TRH. Peak concentrations of 30-157 ng/ml were reached between 5 and 10 min after injection and returned to basal levels between 50 and 120 min (Fig. 3).

Prolactin Concentration in Plasma of Lactating and Non-lactating Possums

Lactation was followed for 174 days after birth in one possum (6058), at which time the young died. Plasma prolactin concentrations were low or undetectable during the first 60 days and then fluctuated from basal concentrations to 45 ng/ml until 120 days. Thereafter concentrations were 50 ng/ml or more until the young was lost (Fig. 4). The two animals (6073, 6076) sampled from day 46 and 72

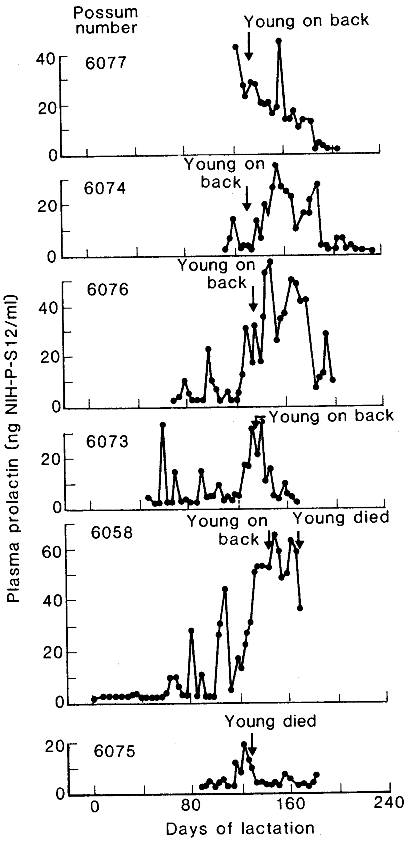

Fig. 4. Plasma prolactin concentrations (ng/ml) in individual lactating possums (n = 6). Animals were caught at different times after the birth of their young and sampled thereafter.

respectively showed similar fluctuations in plasma prolactin until about day 120 (Fig. 4). Thereafter peak concentrations of between 30 and 50 ng/ml were observed until about day 180 in all females which lactated successfully. Beyond day 180 concentrations declined to basal even though the mammary gland was being sucked and the young were still associated with the mother (Fig. 4). Fig. 5 presents a composite profile of prolactin concentrations for these five females throughout lactation and also includes the results for the possums sampled in the following year. There was a significant difference in plasma prolactin concentrations between females with young less than 50 days and females with young of 100–145 days ($5 \cdot 7 \pm 0 \cdot 36$ ng/ml, mean ± s.e.m., $n = 11$, *v.* $30 \cdot 2 \pm 3 \cdot 6$ ng/ml, $n = 24$; $P < 0 \cdot 0005$, one-tailed *t*-test).

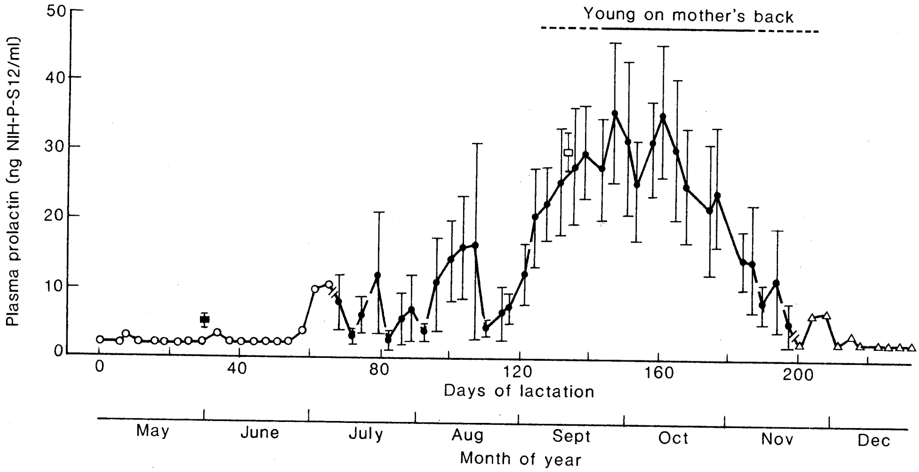

Fig. 5. Mean ± s.e.m. plasma prolactin concentrations for five possums during lactation. From 0 to 65 days, $n = 1$, from 68 to 120 days, $n = 3$, from 120 to 163 days, $n = 5$, from 163 to 194 days, $n = 3$, and from 200 to 233 days, $n = 1$. Single samples from 11 possums with young between 22 and 50 days old (■) and from 24 possums with young between 100 and 145 days old (□) are also shown.

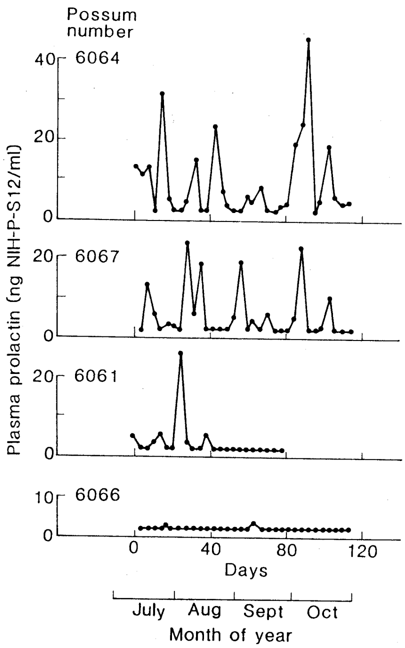

Fig. 6. Plasma prolactin concentrations (ng/ml) in four non-lactating possums sampled between the major and minor breeding seasons from July to October.

In the sixth female (6075), although the pattern of plasma prolactin before 120 days was similar to the other females at this stage of lactation, her young died at 129 days. Thereafter, prolactin concentrations did not change until day 178 when she escaped from captivity (Fig. 4).

Plasma prolactin concentrations in non-lactating females showed no consistent pattern. In two females (6064, 6067) concentrations fluctuated from undetectable amount to more than 20 ng/ml. However, these higher concentrations were not maintained for more than two or three consecutive samples. In the other two animals (6061, 6066) prolactin was undetectable in 17 of 23 plasma samples and in 30 of 32 plasma samples respectively (Fig. 6).

Discussion

The heterologous assay system originally described by McNeilly and Friesen (1978) and validated for the tammar by Hinds and Tyndale-Biscoe (1982) using antiserum 33-9, has now been validated for the possum and tammar using antiserum 33/1-8. Cross-reaction studies with antiserum 33/1-8 have demonstrated that its specificity for marsupial prolactins is similar to antiserum 33-9. Pituitary homogenates of several marsupial species, purified tammar prolactin, and plasma from possums and tammars show parallel displacement to the standard curve for ovine prolactin. Prolactin concentrations determined in quality control plasmas from tammars using antiserum 33-9 or 33/1-8 were not significantly different. Hence any differences in prolactin concentrations between species reflect real differences and are not a result of using different antibodies. Furthermore since TRH induced a significant rise in prolactin in the male and female possums, similar in time course to that seen in eutherian species (Davis and Borger 1972; Convey *et al.* 1972) and the tammar (Hinds and Tyndale-Biscoe 1982) it is reasonable to presume the assay is measuring prolactin.

Although the peak plasma prolactin concentrations (30–50 ng/ml) in possums are lower than in the tammar (>100 ng/ml), and the duration of lactation is shorter (6 months *v.* 9 months) the general pattern closely resembles that described for the tammar (Hinds and Tyndale-Biscoe 1982, 1985). Furthermore, as in the tammar, the marked changes in prolactin concentrations after 60 days correlate well with the rapid increase in the weight of the mammary gland from less than 3 g before 80 days to a maximum of about 12 g by 130 days (Smith *et al.* 1969), the increase in the percentage of protein, fat and total solids in the milk after 65 days (Gross and Bolliger 1959) and the increase in body weight of the young which vacates the pouch at about 120 days. The young, however, remains associated with the female and continues to feed on milk until about day 200–230 (Dunnet 1956; Lyne and Verhagen 1957).

In non-lactating possums there were no consistent changes in plasma prolactin, implying that there are no changes in the secretion of prolactin by the pituitary between the major and minor breeding seasons (July to October). Since sustained increases in plasma prolactin occurred in lactating possums but not in either non-lactating females or in one female after she lost her young, the high concentrations of prolactin in late lactation can probably be attributed to the sucking stimulus of the young, as was demonstrated experimentally for the tammar (Hinds and Tyndale-Biscoe 1985). Thus the unusual pattern of plasma prolactin during lactation first observed in the tammar also occurs in the possum and may be presumed to be a common feature of lactation in marsupials.

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