Aust. J. Biol. Sci., 1981, **34**, 455–61

The Foetoplacental Unit and the Initiation of Lactation in the Rat

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Abstract

Rats were either Caesarean-sectioned, ovariohysterectomized or ovariectomized on day 19 of gestation and the role of prolactin and corticosteroids in the initiation of lactation was studied by administering 2-bromo-α-ergocryptine (CB 154) and prolactin, and by using adrenalectomy and foetectomy. The concentrations of corticosteroids and prolactin in the plasma and the weight and lactose content of the inguinal mammary glands were determined 48 h after Caesarean section, and 24 h after ovariohysterectomy and ovariectomy.

The ranges in concentrations of corticosteroids, prolactin and mammary lactose were 207–348 μg/l plasma, 21·9–65·3 μg/l plasma and 1·21–4·50 mg/g tissue, respectively, in the Caesarean-sectioned, ovariohysterectomized and ovariectomized rats. The administration of CB 154 after either Caesarean section, ovariohysterectomy or ovariectomy decreased the concentration of prolactin to less than 8·8 μg/l. Whereas CB 154 significantly depressed the lactose content of the mammary tissue in Caesarean-sectioned and ovariohysterectomized rats (0·42 and 0·31 mg/g tissue, respectively), no effect was observed in ovariectomized rats (1·39 mg/g tissue). The administration of ovine prolactin reversed the inhibitory effects of CB 154. Furthermore, in two rats which were both ovariectomized and foetectomized, CB 154 did not inhibit lactose accumulation in the mammary tissue (2·02 mg/g tissue).

Adrenalectomy of ovariohysterectomized rats decreased corticosteroid concentrations from 348 to 14 μg/l plasma and the lactose content of mammary tissue from 1·31 to 0·19 mg/g tissue. Whereas adrenalectomy of ovariectomized rats decreased corticosteroid concentrations to 53 μg/l, the lactose content of mammary tissue (1·82 mg/g tissue) remained within the range for ovariohysterectomized rats.

These findings show that hormones produced in late pregnancy by the foetus and placenta can support lactogenesis in the rat in the absence of maternal prolactin and corticosteroids.

Introduction

Previous studies on the hormonal control of the initiation of lactation have shown that hypophysectomized rats demonstrate either a transient or diminished lactogenic response in late pregnancy (Selye *et al.* 1933; Yokoyama *et al.* 1969). This response was absent if the foetus and placenta were also removed (Collip *et al.* 1933; Yokoyama *et al.* 1969). Mammotrophic properties have been attributed to the placenta (see reviews by Forsyth 1974; Matthies 1974), and rat chorionic mammotrophin (placental lactogen) has been isolated and characterized (Robertson and Friesen 1975). The concentration of placental lactogen, measured in the maternal plasma using a radioreceptor assay, is elevated during mid-pregnancy and for 4–5 days prior to parturition (Shiu *et al.* 1973). In addition, corticosteroids are transported from the foetus to the maternal blood compartment of adrenalectomized rats from day 18 of pregnancy until term (Milkovic *et al.* 1973; Petropoulas and Lau 1973; Dupouy *et al.* 1975).

Since the rat requires both corticosteroid and a lactogen in order to complete the lactogenic complex (Denamur 1971), the present study was undertaken to investigate the capacity of the foetoplacental complex to supply the hormones required for the initiation of lactation in rats.

Materials and Methods

Ovine prolactin (25 i.u./mg) was provided by the Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A. (NIH-P-S 10) and administered subcutaneously in 0·9% (w/v) sodium chloride at the rate of 50 i.u. per 12 h per rat. 2-Bromo-α-ergocryptine mesylate (CB 154) was provided by Sandoz Ltd, Basel, Switzerland, and was administered subcutaneously in 20% (v/v) ethanol at 1·0 mg per rat.

Female rats (*Rattus norvegicus*, Wilstar strain) were maintained as described previously (Nicholas and Hartmann 1981*a*). All procedures were carried out on the rats between 1600 and 1800 h. Rats were housed individually after surgery and blood samples were collected from the jugular vein within 20 s of death. Lactose content of the mammary gland was determined as previously described (Nicholas and Hartmann 1981*a*). The concentration of corticosteroids in the plasma was measured by the competitive protein-binding assay of Martin *et al.* (1977), and the concentration of prolactin by a double antibody radioimmunoassay using reagents supplied by the Rat Pituitary Hormone Program, National Institute of Arthritis and Metabolic Diseases (Nicholas and Hartmann 1981*b*).

Rats were divided into three groups and treated as follows:

(1) *Caesarean-sectioned group.* All rats in this group were Caesarean-sectioned (the foetuses and placentae removed) at day 19 of gestation. Control rats were untreated after Caesarean section and the other rats were administered either CB 154, CB 154 plus prolactin, or prolactin. All rats were killed 48 h after surgery and the concentrations of lactose in the mammary glands and corticosteroids and prolactin in the plasma were determined.

(2) *Ovariohysterectomized group.* All rats in this group were ovariohysterectomized (ovaries and uterine horns removed) at day 19 of gestation. Control rats were untreated after ovariohysterectomy and the other rats were administered either CB 154, CB 154 plus prolactin, or prolactin. An additional group of rats were both ovariohysterectomized and adrenalectomized on day 19 of gestation. All rats were killed 24 h after surgery, and the concentrations of lactose in the mammary glands and corticosteroid and prolactin in the plasma were determined.

(3) *Ovariectomized group.* All rats in this group were ovariectomized on day 19 of gestation. Control rats were then untreated while the other rats were either administered CB 154 or adrenalectomized. An additional two rats were both ovariectomized and foetectomized (the foetuses removed and the placentae left *in situ*). The success of this latter procedure was assessed by the appearance of viable placentae 24 h after surgery. The mean weight of the placentae of control rats was 0·40 ± 0·02 g (mean ± s.e.m.), while the placentae from the two rats which were successfully foetectomized weighed 0·48 and 0·41 g respectively. All rats were killed 24 h after surgery and the concentrations of lactose in the mammary glands and of corticosteroid and prolactin in the plasma were determined.

Results

Caesarean-sectioned Rats

Single administration of CB 154 to Caesarean-sectioned rats resulted in a 90% decrease in the concentration of lactose in the mammary gland when compared with control rats 48 h after surgery (Table 1). In contrast the concentration of lactose in the mammary tissue from animals receiving both CB 154 and prolactin decreased by 32% while the rats given prolactin alone had lactose concentrations 48% above control values. The concentration of prolactin in CB 154-treated rats was significantly lower than in the control animals (Table 1).

Ovariohysterectomized Rats

The accumulation of lactose in the mammary gland of rats ovariohysterectomized and either adrenalectomized or treated with CB 154 was suppressed by 85 and 76%, respectively (Table 2). No inhibition was apparent when prolactin was administered

Table 1. Weight and lactose concentration of the mammary gland and the concentration of corticosteroids and prolactin in plasma of rats 48 h after Caesarean section at day 19 of gestation

Individual rats received one of the following treatments after Caesarean section: control, CB 154, CB 154 plus prolactin, or prolactin. Each value represents the mean ± s.e.m., and the values obtained for each parameter were analysed by one-way analysis of variance and least significant difference tests. Mean values underlined by the same line are not significantly different at $P < 0{\cdot}05$

Treatment	No. of observations	Mammary gland Weight (g/200 g body wt)	Mammary gland Lactose (mg/g tissue)	Plasma corticosteroids (μg/l)	Plasma prolactin (μg/l)
1. Control	5	2·85±0·07	4·50±0·20	321±42	21·9±6·1
2. CB 154	7	1·93±0·08	0·42±0·03	417±58	7·7±2·8
3. CB 154 plus prolactin	4	3·03±0·13	3·31±0·29	—	—
4. Prolactin	4	3·11±0·21	6·67±0·55	—	—
Significance		2 1 3 4	2 1 3 4		

Table 2. Weight and lactose concentration of the mammary gland and the concentration of corticosteroids and prolactin in plasma of rats 24 h after ovariohysterectomy at day 19 of gestation

Individual rats received one of the following treatments after ovariohysterectomy: control, CB 154, CB 154 plus prolactin, prolactin or adrenalectomy. Each value represents the mean ± s.e.m., and the values obtained for each parameter were analysed by one-way analysis of variance and least significant difference tests. Mean values underlined by the same line are not significantly different at $P < 0{\cdot}05$

Treatment	No. of observations	Mammary gland Weight (g/200 g body wt)	Mammary gland Lactose (mg/g tissue)	Plasma corticosteroids (μg/l)	Plasma prolactin (μg/l)
1. Control	7	2·34±0·12	1·31±0·08	348±37	38·4±6·8
2. CB 154	9	1·82±0·09	0·31±0·05	304±68	8·8±1·4
3. CB 154 plus prolactin	4	2·35±0·15	1·60±0·16	—	—
4. Prolactin	3	2·29±0·04	2·63±0·37	—	—
5. Adrenalectomy	5	1·29±0·11	0·19±0·05	14±2	47·6±18·1
Significance		1 2 3 4 5	5 2 1 3 4	5 1 2	2 1 5

with CB 154, and when prolactin alone was given there was a 50% increase in the concentrations of lactose in the mammary tissue. The plasma concentrations of corticosteroids and prolactin were significantly depressed in rats 24 h after adrenalectomy and treatment with CB 154, respectively (Table 2).

Ovariectomized Rats

The concentration of lactose in the mammary gland from ovariectomized rats administered CB 154 (1·39 mg/g tissue) was not significantly different from untreated rats (1·21 mg/g tissue), whereas the concentration of prolactin in the plasma was significantly depressed (Table 3). The two rats which were ovariectomized, foetectomized, and administered CB 154 had low plasma prolactin levels but elevated concentrations of lactose in the mammary tissue (Table 3). The concentrations of lactose in the mammary gland of ovariectomized and adrenalectomized rats were not lower than the values for control rats. However, the concentration of corticosteroids in the plasma of these animals had declined to low levels by 24 h after adrenalectomy. The concentration of prolactin in the plasma of these rats was not significantly different from control rats.

Table 3. Weight and lactose concentration of the mammary gland and the concentration of corticosteroids and prolactin in plasma of rats 24 h after ovariectomy at day 19 of gestation

Individual rats received one of the following four treatments after ovariectomy: control, CB 154, adrenalectomy, or foetectomy plus CB 154. Each value represents the mean ±s.e.m., and the values obtained for each parameter were analysed by one-way analysis of variance and least significant difference tests. Mean values underlined by the same line are not significantly different at $P < 0.05$

Treatment	No. of observations	Mammary gland		Plasma corticosteroids (μg/l)	Plasma prolactin (μg/l)
		Weight (g/200 g body wt)	Lactose (mg/g tissue)		
1. Control	6	2·26±0·08	1·21±0·35	207±45	65·3±17·5
2. CB 154	8	2·06±0·12	1·39±0·18	286±44	7·9±2·7
3. Adrenalectomy	10	2·02±0·07	1·82±0·43	53±6	50·4±9·7
4. Foetectomy plus CB 154	2	2·16, 2·11	2·02, 2·19	112, 66·4	3·2, 5·0
Significance		1 2 3 4	1 2 3 4	3 4 1 2	2 4 1 3

Discussion

The requirement for prolactin and corticosteroids in the lactogenic complex of rats has been demonstrated previously in hypophysectomized animals (Bintarningshi *et al.* 1958; Lyons *et al.* 1958; Cowie and Lyons 1959; Abraham *et al.* 1960). However, Kuhn (1969) has drawn attention to the care required in the interpretation of this type of experiment since lactogenesis can occur in hypophysectomized pregnant rats (Selye *et al.* 1933; Yokoyama *et al.* 1969) unless the foetuses and placentae are also removed (Collip *et al.* 1933; Yokoyama *et al.* 1969). In the present study administration of CB 154, which specifically inhibits the secretion of prolactin but not growth hormone from the pituitary (see del Pozo and Flückiger 1973), to ovariohysterectomized and Caesarean-sectioned rats suppressed lactose concentrations in the mammary gland by 77 and 90% respectively (Tables 1 and 2). Furthermore, the concentration of lactose was either completely or partially restored by the administration of prolactin (Table 1), demonstrating the importance of prolactin in the initiation of lactation in the rat.

The requirement for maternal adrenal corticosteroids in the lactogenic complex was demonstrated in late pregnant adrenalectomized rats deprived of the foetoplacental unit (Table 2). The decline in the concentration of corticosteroids in the plasma to low levels by 24 h after adrenalectomy was associated with a significantly depressed concentration of lactose in the mammary tissue and a lower wet weight of the mammary gland (Table 2). Similarly, Liu and Davis (1969) demonstrated a diminished secretion of casein-like protein and a decrease in the concentration of RNA in the mammary gland of rats ovariectomized at day 15 of gestation 24 h after adrenalectomy. Normal milk secretion was restored with the administration of either corticosterone or cortisol. These findings show that, in the absence of the foetus and placenta, the rat needs both corticosteroids and prolactin for lactogenesis.

In contrast to the rabbit (Meites *et al.* 1963) the administration of prolactin to pregnant rats does not induce lactation (Talwalker *et al.* 1961; Kuhn 1969). Simpson *et al.* (1973) have found that the increased plasma prolactin concentration resulting from the daily administration of perphenazine during late pregnancy in the rat did not significantly change the concentration of mammary lactose, glucose, RNA, DNA or the RNA : DNA ratio. However, we have found that the administration of ovine prolactin to rats immediately after lactogenesis had been induced in late pregnancy by either Caesarean section or ovariohysterectomy, significantly increased the accumulation of lactose in the mammary gland above control values (Tables 1 and 2). In this connection it was observed that a decline in progesterone levels to below 10·0 μg/l in late pregnancy and after either Caesarean section or ovariohysterectomy was followed by a rapid increase in the concentrations of prolactin in the plasma and of lactose in the mammary gland (Nicholas and Hartmann 1981*b*). Taken collectively, these data suggest that mammary tissue can respond to prolactin only after the removal of progesterone.

The capacity of the rat to support lactogenesis in the absence of prolactin was tested in ovariectomized and Caesarean-sectioned rats in which the concentrations of prolactin was suppressed by CB 154. The accumulation of lactose was not inhibited in ovariectomized rats, suggesting that the foetus or placenta or both could supply lactogenic hormones to replace prolactin in these rats. Lactogenesis occurred in ovariectomized, foetectomized rats treated with CB 154, which suggests that the lactogenic factor can be derived from the placenta, and is probably placental lactogen. These findings are in agreement with the results of Ray *et al.* (1955), who demonstrated that placental extracts have lactogenic properties when administered together with hydrocortisone acetate to hypophysectomized and ovariectomized rats.

The potential for transfer of corticosteroids from the foetus (Petropoulas and Lau 1973; Dupouy *et al.* 1975) and the placenta (Petropoulas and Lau 1973) to the maternal circulation in late pregnancy has become well established. However, whether the foetus is capable of providing sufficient adrenal corticosteroids to support lactogenesis remains equivocal. In contrast to the results of Dupouy *et al.* (1975), both the present findings (Tables 2 and 3) and the observations of Petropoulas and Lau (1973) show that the concentrations of corticosteroids in the plasma decrease to low values 24 h after adrenalectomy. Since the accumulation of lactose in the mammary tissue was impaired in ovariohysterectomized rats (Table 2) but not in ovariectomized rats (Table 3), it is clear that lactogenesis can proceed in the presence of low concentrations (48–65 μg/l) of corticosteroids derived from the foetoplacental complex.

Previous findings (Nicholas and Hartmann 1981*b*) have shown that there is no change in the concentration of either total corticosteroids or CBG capacity in the plasma of rats during the latter stages of pregnancy, suggesting that the level of unbound (active) hormone is sufficient for lactation to ensue. Although CBG capacity was not measured in the present study, the results (Tables 1, 2 and 3) suggest that the concentration of corticosteroids in the plasma of the late pregnant rat was far greater than that required for the initiation of lactation.

Yokoyama *et al.* (1969) have shown that the concentrations of lactose in the mammary gland from late pregnant rats after hypophysectomy was *c.* 65% less than those of sham-operated animals. The present findings show that the initiation of lactation proceeded unimpaired in ovariectomized rats which were subsequently adrenalectomized or administered CB 154 (Table 3). However, Pepe and Rothchild (1972) have reported a prolonged elevation of progesterone in hypophysectomized pregnant rats. Since a decline in the concentration of plasma progesterone to low levels in late pregnancy is a prerequisite for a rapid increase in the synthesis of lactose (Nicholas and Hartmann 1981*a*), the high levels of progesterone which accompany hypophysectomy may account for the diminished lactogenic response described by Yokoyama *et al.* (1969).

These findings question the hypothesis that completion of the lactogenic complex in terms of either prolactin or corticosteroids is an important stimulus for lactogenesis in the rat (Cowie 1969). The ability of the foetoplacental complex to supply the hormones necessary for the initiation of lactation indicates that the requirements for the lactogenic complex are satisfied before the decline in progesterone in late pregnancy in the rat. Furthermore, the data is in agreement with a recent experiment (Nicholas and Topper 1980) which demonstrated that progesterone directly inhibits lactogenesis in the rat.

Acknowledgments

We thank Jane Wilson and Helen Nottage for skilled technical assistance. The work was supported by the National Health and Medical Research Council of Australia.

References

Abraham, S., Cady, P., and Chaikoff, I. L. (1960). Glucose acetate metabolism and lipogenesis in mammary glands of hypophysectomized rats in which lactation was hormonally induced. *Endocrinology* **66**, 208–88.

Bintarningsih, Lyons, W. R., Johnson, R. E., and Li, C. H. (1958). Hormonally-induced lactation in hypophysectomized rats. *Endocrinology* **63**, 540–8.

Collip, J. B., Selye, H., and Thompson, D. L. (1933). Further observations on the effect of hypophysectomy on lactation. *Proc. Soc. Exp. Biol. Med.* **30**, 913.

Cowie, A. T., and Lyons, W. R. (1959). Mammogenesis and lactogenesis in hypophysectomized, ovariectomized and adrenalectomized rats. *J. Endocrinol.* **19**, 29–32.

Cowie, A. T. (1969). The initiation of milk secretion at parturition. In 'Lactogenesis'. (Eds M. Reynolds and S. J. Folley.) pp. 157–69. (University of Pennsylvania Press: Pennsylvania.)

Denamur, R. (1971). Hormonal control of lactogenesis. *J. Dairy Res.* **38**, 237–64.

Dupouy, J. P., Coffigny, H., and Magre, S. (1975). Maternal and foetal corticosterone levels during late pregnancy in rats. *J. Endocrinol.* **65**, 347–52.

Forsyth, I. A. (1974). The comparative study of placental lactogenic hormones: A review. In 'Lactogenic Hormones, Foetal Nutrition and Lactation'. (Eds J. B. Josimovich, M. Reynolds and E. Cobo.) pp. 49–67. (John Wiley & Sons: New York.)

Kuhn, N. J. (1969). Progesterone withdrawal as the lactogenic trigger in the rat. *J. Endocrinol.* **44**, 39–54.

Liu, T. M. Y., and Davis, J. W. (1969). Induction of lactation by ovariectomy of pregnant rats. *Endocrinology* **80**, 1043–50.

Lyons, W. R., Li, C. H., and Johnson, R. E. (1958). Hormonal control of mammary growth and lactation. *Rec. Progr. Horm. Res.* **14**, 219–54.

Martin, C. E., Cake, M. H., Hartmann, P. E., and Cook, I. F. (1977). Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. *Acta Endocrinol. (Copenhagen)* **84**, 167–76.

Matthies, D. L. (1974). Placental peptide hormones affecting fetal nutrition and lactation: Effects of rodent chorionic mammatrophin. In 'Lactogenic Hormones, Foetal Nutrition and Lactation'. (Eds J. B. Josimovich, M. Reynolds and E. Cobo.) pp. 49–67. (John Wiley & Sons: New York.)

Meites, J., Hopkins, T. F., and Talwalker, P. K. (1963). Notes and comments: Induction of lactation in pregnant rabbits with prolactin, cortisol acetate or both. *Endocrinology* **73**, 261–4.

Milkovic, S., Milkovic, K., and Davrovic, J. (1973). The initiation of foetal adrenocorticotrophic activity in the rat. *Endocrinology* **92**, 380–4.

Nicholas, K. R., and Hartmann, P. E. (1981*a*). Progesterone control of the initiation of lactose synthesis in the rat. *Aust. J. Biol. Sci.* **34**, 435–43.

Nicholas, K. R., and Hartmann, P. E. (1981*b*). Progressive changes in plasma progesterone, prolactin and corticosteroids in plasma during late pregnancy and the initiation of lactose synthesis in the rat. *Aust. J. Biol. Sci.* **34**, 445–54.

Nicholas, K. R., and Topper, Y. J. (1980). Enhancement of α-lactalbumin-like activity in mammary explants from pregnant rats in the absence of exogenous prolactin. *Biochem. Biophys. Res. Comm.* **94**, 1424–31.

Pepe, G. J., and Rothchild, I. (1972). The effect of hypophysectomy on day 12 of pregnancy on the serum progesterone level and time of parturition in the rat. *Endocrinology* **91**, 1380–91.

Petropoulos, E. A., and Lau, C. (1973). Foetoplacental contribution to the maternal corticosteroid pool in Long-Evans rats. *J. Endocrinol.* **59**, 183–4.

Pozo, E. del, and Flückiger, E. (1973). Human prolactin. In 'Proceedings of the International Symposium on Human Prolactin.' (Eds J. L. Pasteels and C. Robyn.) p. 291. (Excerpta Medica: Amsterdam.)

Ray, E. W., Averill, S. C., Lyons, W. R., and Johnson, R. E. (1955). Rat placental hormonal activities in comparison to those of pituitary mammatrophin. *Endocrinology* **56**, 359–73.

Robertson, M. C., and Friesen, H. G. (1975). The purification and characterization of rat placental lactogen. *Endocrinology* **97**, 621–9.

Shiu, R. P. C., Kelly, P. A., and Friesen, H. G. (1973). Radioreceptor assay for prolactin and other lactogenic hormones. *Science* **180**, 968–71.

Selye, H., Collip, J. B., and Thompson, D. L. (1933). Anterior pituitary and lactation. *Proc. Soc. Exp. Biol. Med.* **30**, 588–9.

Simpson, A. A., Simpson, M. H. W., and Kulkarni, P. N. (1973). Effect of perphenazine during late pregnancy on prolactin production and lactogenesis in the rat. *J. Endocrinol.* **57**, 431–6.

Talwalker, P. K., Nicholl, C. S., and Meites, J. (1961). Induction of mammary secretion in pregnant rats and rabbits by hydrocortisone acetate. *Endocrinology* **69**, 802–8.

Yokoyama, A., Shinde, Y., and Ota, K. (1969). Endocrine control of changes in lactose content of the mammary glands in rats shortly before and after parturition. In 'Lactogenesis'. (Eds M. Reynolds and S. J. Folley.) pp. 65–71. (University Pennsylvania Press: Pennsylvania.)

Manuscript received 15 September 1980, accepted 13 May 1981