INFLUENCE OF ENDOGENOUS SOMATOSTATIN ON LACTATION IN RATS

S. S. Sun, K. H. Myung[1] and G. S. G. Spencer[2]

Chonnam National University, Kwangju 500-757, Korea

Summary

The role of endogenous somatostatin on lactation in rats was examined by passive immuno-neutralization of Wistar rats. In one study, the rats were given either immunoglobulin raised in sheep against somatostatin, or non-specific sheep immunoglobulins by daily s.c. injection throughout pregnancy. In second series of experiments, the dams were passively immunized by i.p. injection from parturition through the first two weeks of lactation. The growth of the pups was recorded by weighing every second day, and the milk yield calculated from the pup weight and weight gain. Immunoneutralization of maternal somatostatin during pregnancy had a slight effect ($p < 0.05$) on the mean birth weight of the pups but no subsequent effect on postnatal growth rate of the pups or milk yield (25.32 ± 0.88 g/day) compared with young control rats given normal sheep serum (25.55 ± 1.04 g/day). Similarly, passive immunization against somatostatin during lactation (21.96 ± 1.57 g/day) also did not affect milk yields compared with controls (24.85 ± 1.03 g/day). These data do not support a significant role for endogenous somatostatin in regulating milk production in lactating rats.

(**Key Words** : Immunoneutralization, Somatostatin, Lactation)

Introduction

It is well established that, in ruminants at least, growth hormone (GH) plays an important role in regulating milk production (Baumann et al., 1985). It has been reported that immunoneutralization of rat GH during pregnancy in rats is associated with a failure to lactate normally (Spencer et al., 1994) providing evidence that GH can also influence lactation in rats. However the study did not differentiate between prepartum effects on mammogenesis and direct postpartum influences on mammary gland function as measured by milk production.

Although reducing GH levels during lactation by active immunization against growth hormone releasing factor (GRF) has no effect on milk yields in cattle (Moore et al., 1992), immunization against growth hormone release inhibiting hormone (somatostatin), may increase GH, and has been shown to increase milk production in goats (Spencer et al., 1985; Garssen et al., 1987) and sheep (Sun et al., 1990).

The present studies were undertaken to investigate whether endogenous somatostatin has a role in regulating lactation in rats. This was done by the use of passive immunoneutralization of somatostatin during pregnancy or during lactation.

Materials and Methods

Preparation of antisera

Anti-somatostatin sera were raised in sheep to a somatostatin-14-human serum globulin conjugate (Spencer et al., 1983). The IgG fraction from this serum, and from a pool of normal sheep serum, was prepared by multiple Na_2SO_4 precipitation (Kekwick, 1940).

Animals

Wistar rats were used in this study. They were caged individually throughout pregnancy and lactation under standard conditions of 12 h : 12 h light : dark, at an ambient temperature of 22℃ with laboratory rat food (NRM Feeds Ltd., New Zealand) and water available *ad libitum*.

Immunization throughout pregnancy

On the day of proestrus (as determined by daily vaginal smears), the rats were mated with males. The

[1]Address reprint requests to Dr. K. H. Myung, Department of Animal Science, Chonnam National University, Kwangju 500-757, Korea.

[2]Ruakura Agricultural Centre, Hamilton, New Zealand.

Received September 29, 1995
Accepted January 22, 1996

following morning the rats were checked for insemination by the presence of sperm in the vaginal swab, and this was called day 1 of pregnancy. Pregnancy was confirmed by daily smears for the next 8 days to check for the absence of cycling.

On day 2 of pregnancy, the rats received either 1 ml anti-somatostatin serum s.c. (n = 6), or 1 ml normal sheep serum s.c. (n = 7). The appropriate treatment was repeated daily throughout pregnancy. The rats were allowed to continue through to term and deliver their young naturally. Within 12 hours of birth the pups were weighed and sexed. Litter sizes were adjusted to 10 ± 2 and the pups were left with the mothers throughout. Milk yield was estimated using the formula described by Sampson and Jansen (1984) over the period 4-14 days of age.

The anti-somatostatin serum used in these experiments was specific for somatostatin and was capable of binding 51ng somatostatin/ml of iodinated Tyr[1] somatostatin *in vitro*.

Immunization during lactation

Seventeen female rats were used in this study. Within 24 hours of birth, litter sizes were adjusted to 8 and the mothers were given 1 ml of anti-somatostatin IgG (capable of neutralizing 51ng of somatostatin *in vitro*), or a similar volume of IgG purified from normal sheep serum. Eleven (6 anti-somatostatin and 5 controls) dams were in their first lactation, and 3 anti-somatostatin and 3 controls were in their second lactation.

The weights of the pups were recorded every other day throughout the trial. On day 10-11 of lactation an estimate of milk yield was made in some rats using the separation technique. The mother and pups were weighed and then the pups were removed from the mother. After 3 hours of separation, the bladder of the pups was emptied by gentle pressure and the pups were weighed again and returned to the mothers. Three hours later, mothers and pups were weighed again, and the increase in total litter weight was taken to be equal to the amount of milk ingested.

Calculation of milk yield was also made using the formula described by Sampson and Jansen (1984) over the period 5-11 days (first pregnancy) and 4-10 days (second pregnancy).

Statistical analysis of milk yield differences were made by t-test.

Results

Immunization during pregnacy

There was no difference in the mean maternal weights between the immunized and control animals (355 g and 376 g, respectively), but the mean birth weight of the pups of the anti-somatostatin treated rats was slightly heavier than that of the controls (6.11 ± 0.09 g and 5.78 ± 0.08 g, respectively) while postnatal growth rates over the study period were not significantly different. This was reflected in no significant difference in calculated milk yield between the treated and control rats.

TABLE 1. BIRTH WEIGHT AND AVERAGE DAILY WEIGHT GAIN (ADWG) OF PUPS BORN OF WISTAR MOTHERS, AND DAM'S MILK YIELD FOLLOWING DAILY TREATMENT THROUGHOUT PREGNANCY WITH 1 ml ANTI-SOMATOSTATIN SERUM OR 1 ml NORMAL SHEEP SERUM (CONTROL)

	Control	Anti-somatostatin
Birth Wt (g)	5.78 ± 0.09	6.11 ± 0.09*
ADWG (g/d)	1.62 ± 0.09	1.60 ± 0.06
Milk yield (g/d)	25.55 ± 1.04	25.32 ± 0.88

* $p < 0.05$.

Immunization during lactation

The mean weight of mothers in the two groups were not significantly different at parturition (360 ± 16.6 g and 349 ± 12.5 g) and there was no significant difference in body weight changes between the groups throughout the period of study (17.2 ± 4.0 g and 13.8 ± 10.7 g).

There was no significant difference between milk yields from mothers in the first and second lactations, and these data were pooled.

The mean birth weights of the pups from treated and control dams (6.98 ± 0.08 g and 6.32 ± 0.08 g) were not significantly different, and neither was body weight gain of the pups of the anti-somatostatin treated mothers (1.86 ± 0.11 g/day) different from that of control pups (1.98 ± 0.14 g/day).

Using the formula for estimating milk yields used by Sampson and Jansen (1984) there was no significant effect of passive immunization of somatostatin of lactating mothers on milk yield over the period from 5-11 days postpartum (24.85 ± 1.03 g/day and 21.96 ± 1.7 g/day for control and anti-somatostatin rats, respectively).

Estimates of milk yield obtained by the separation technique also were not significantly different between the groups (44.55 ± 4.55 g/d and 35.60 ± 2.39 g/d).

TABLE 2. BIRTH WEIGHT AND AVERAGE DAILY WEIGHT GAIN (ADWG) OF PUPS BORN OF WISTAR MOTHERS, AND DAM'S MILK YIELD FOLLOWING DAILY TREATMENT WITH 1 ml ANTI-SOMATOSTATIN SERUM OR 1 ml NORMAL SHEEP SERUM (CONTROL) UNTIL DAYS 14 POSTPARTUM

	Control	Anti-somatostatin
Birth Wt (g)	6.32 ± 0.08	6.98 ± 0.08
ADWG	1.98 ± 0.14	1.86 ± 0.11
Milk yield (g/d)[A]	24.85 ± 1.03	21.96 ± 1.57
Milk yield (g/d)[B]	44.55 ± 4.55	35.60 ± 2.39

[A] : Estimated milk yield using the formula described by Sampson and Jansen (1984) over the period 4-14 days of age.

[B] : Estimated milk yield using separation technique (Madon et al., 1986) on day 10-11 of lactation.

Discussion

It has previously been reported that passive immunization of somatostatin in pregnant rats can result in increased postnatal growth of the offspring (Spencer et al., 1994). Whether this was a result of the increased size at birth, increased milk production, or alteration of pup hormonal status was not clear.

Direct administration of anti-somatostatin serum to rat pups has been shown to alter growth and hormone levels in the pups (Robinson et al., 1993), and antibodies to somatostatin, ingested with colostrum and milk, increased growth of lambs (Westbrook et al., 1994). These data may indicate an effect of ingested antibodies having direct effects on pup hormones and growth. However, Farmer et al. (1991) could find no effect on neonatal hormones in piglets following perinatal manipulation.

It is well known that increased birth weight is associated with increased postnatal growth. The increase in fetal size seen with immunization against somatostatin during pregnancy in this study concurs with earlier observations in rats (Spencer et al., 1994) and sheep (Spencer, 1986). However, other studies on active immunization against somatostatin during pregnancy have not been able to show significant differences in birth weights of the young (van Kessel et al., 1990; Farmer et al., 1991). One explanation for the lack of consistent results on birth weight may be that antibody generation does not necessarily lead to immunoneutralization of somatostatin, and data on the neutralizing efficacy in earlier trials were not reported. Another possibility is that endogenous somatostatin may be unable to alter the production of placental somatotropin or its variants, which may play a significant role during pregnancy (Caufriez et al., 1993).

In these experiments we could find no evidence that reduced maternal plasma somatostatin influenced milk production in rats. This contrasts with the results obtained in goats and sheep (Spencer et al., 1985; Garssen et al., 1987; Sun et al., 1990). This may be because somatotropin is less galactopoietic in rats than in ruminants, being only of major importance in situations of prolactin insufficiency (Madon et al., 1986).

From these data we concluded that immunization against somatostatin, although enhancing milk yields in ruminants, does not influence on milk production in rats.

Conclusion

Immunoneutralization of maternal somatostatin during lactation or during pregnancy did not affect milk yield. This might mean that endogenous somatostatin of lactating rats does not have a significant role for regulating milk production.

Literature Cited

Baumann D. E., P. J. Eppard, M. J. Degeeter and G. M. Lanza. 1985. Responses of high-producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. Journal of Dairy Science 68:1352-1362.

Caufriez, A., F. Franuenne, G. Hennen and G. Copinschi. 1993. Regulation of maternal IGF-1 by Placental GH in normal and abnormal human pregnancies. American Journal of Physiology 265:E572-577.

Farmer, C., G. Pelletier and D. Peticlerc. 1991. Immunization against somatostatin and/or injection of growth hormone releasing factor in pregnant sows : Is there a potential ? Bulletin Agriculture Canada, Research Branch. No. 14, 1-2.

Garssen, G. J., G. S. G. Spencer and A. M. A. W. Welling. 1987. Active immunization against somatostatin increases milk yield in goats. Netherlands Journal of Agricultural Science 35:84-86.

Kekwick, A. 1940. The serum proteins in multiple myelomatosis. Biochemical Journal 34:1248-1257.

Madon, R. J., D. M. Ensor, C. H. Knight and D. J. Flint. 1986. Effects of an antiserum to rat growth hormone on lactation in the rat. Journal of Endocrinology 111:117-123.

Moore K. L., J. D. Armstrong, R. M. Harvey and E. P. Heimer. 1992. Effect of active immunization against growth hormone releasing factor on concentrations of somatotropin and insulin-like growth factor-1 in lactating beef cows. Domestic Animal Endocrinology 9:125-139.

Robinson, G., G. S. G. Spencer, C. G. Berry, P. M. Dobbie, S. C. Hodgkinson and J. J. Bass. 1993. Evidence of a role for growth hormone, but not for insulin-like growth factor-Ⅰ or -Ⅱ, in growth of the neonatal rat. Biology of the Neonate 64:158-165.

Sampson, D. A. and G. R. Jansen. 1984. Measurement of milk yield in the lactating rat from pup weight and weight gain. Journal of Pediatric Gastroenterology and Nutrition. 3:613-617.

Spencer, G. S. G., G. J. Garssen and I. C. Hart. 1983. A novel approach to growth promotion using auto-immunization against somatostatin. Ⅰ. Effects on growth hormone levles in lambs. Livestock Production Science 10:25-37.

Spencer, G. S. G., G. J. Garssen and A. M. A. W. Welling. 1985. Increased milk production by goats following immunization against somatostatin. Animal Production. 40:572-573.

Spencer G. S. G. 1986. Immuno-neutralization of somatostatin and its effects on animal production. Domestic Animal Endocrinology 3:55-68.

Spencer, G. S. G., G. K. Robinson, C. J. Berry and P. M. Dobbie. 1994. Alteration of maternal growth hormone levels during pregnancy influences both fetal and postnatal growth in rats. Biology of the Neonate 66:112-118.

Sun, X. Y., S. E. Sindaur, P. C. Wynn and G. H. McDowell. 1990. Immunization against somatotropin release inhibiting factor increases milk yield in ewes. Australian Journal of Agricultural Research 41:393-400.

van Kessel, A. G., R. S. Korchinski, C. H. Hampton and B. Laarveld. 1990. Effect of immunization against somatostatin in the pregnant ewe on growth and endocrine status of the neonatal lamb. Domestic Animal Endocrinology 7:217-228.

Westbrook, S. L., A. Shulkes and G. H. McDowell. 1994. Antibodies to somatotropin release inhibiting factor (SRIF) alters appetite and secretion of gastrin in suckling lambs. Proceeding of the Nutrition Society Physiology 3:293.