The role of endogenous insulin-like growth factors-1 and -2 on lactation in rats

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ABSTRACT

The role of endogenous insulin-like growth factor (IGF)-1 and -2 on lactation in rats was examined by passive immuno-neutralization of Wistar rats. In one study, the rats were given immunoglobulins raised in sheep against either IGF-1, IGF-2 or non-specific sheep immunoglobulins by daily s.c. injection throughout pregnancy. In a 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 either IGF-1 or IGF-2 in the mother during pregnancy had no effect on the mean birth weight or postnatal growth rate of the pups. Milk yield from rats treated during pregnancy (21.5 ± 1.2 and 20.3 ± 0.9 g/day for anti-IGF-1 and anti-IGF-2, respectively) compared with control rats given normal sheep serum (21.9 ± 1.1 g/day) was also not significantly different. Similarly, passive immunization against either IGF-1 (22.2 ± 0.9 g/d) or IGF-2 (18.0 ± 2.0 g/d) during lactation also did not affect milk yields compared with controls (24.9 ± 1.0 g/day). These data do not support a significant role for endogenous IGFs in regulating milk production in lactating rats.

Keywords: insulin-like growth factors; immunoneutralization; lactation; pregnancy; rat.

INTRODUCTION

It is well established that, in ruminants at least, growth hormone (GH) plays an important role in regulating milk production. Various studies have also demonstrated a role for GH during lactation in rats (Madon *et al.*, 1986) but it is not clear whether the galactopoietic effects of GH are mediated by the insulin-like growth factors (IGFs). Prosser *et al.* (1990) have found that infusion of IGF-1 into a pudic artery in goats may increase milk production in the infused gland although others have reported no effect of IGF-1 on lactation in rats (Flint *et al.*, 1993).

It has been previously reported that the offspring from rats immunized against IGF-1 during pregnancy have a slower rate of growth postnatally (Berry *et al.*, 1993) and it was postulated that this may have been the result of retarded mammary gland development or a direct effect of reduced maternal plasma IGF-1 levels on galactopoiesis. The present experiments investigate the role of endogenous IGF-1 and IGF-2 during pregnancy or during lactation on milk yield in rats by using specific immuno-neutralization of the IGFs.

MATERIALS AND METHODS

Preparation of antisera

Antisera were raised in sheep to IGF-1 or IGF-2 conjugated to ovalbumin. The IgG fraction from the sera, and from a pool of normal sheep serum, was prepared by Na_2SO_4 precipitation. Differing efficiencies of purification resulted in slight differences in the binding capacities of the preparations used in these two sets of experiments. Both sets of antisera were tested for immunoneutralizing capacity and the binding capacities are detailed in the appropriate sections.

Animals

Wistar rats were caged individually throughout pregnancy and lactation under standard conditions of 12h:12h light:dark, at 22°C with laboratory rat food (NRM Feeds Ltd., NZ) and water available *ad libitum*.

Immunization throughout pregnancy

The anti-IGF-1 serum used in this experiment was specific for IGF-1 and was capable of binding 7.5 µg/ml of iodinated IGF-1 *in vitro*. This antiserum was demonstrated to have immunoneutralizing capacity by its ability to inhibit labelled IGF-1 binding to kidney membrane receptors. Similarly, the anti-IGF-2 serum showed <1% cross-reactivity with insulin or IGF-1 and bound 5.6 µg/ml of iodinated IGF-2 *in vitro*.

On day 2 of pregnancy, the rats received a sub-cutaneous injection of either 1 ml anti-IGF-1 IgG (n=8), 1 ml of anti-IGF-2 IgG (n=8) or 1 ml normal sheep IgG (n=12). The appropriate treatment was repeated daily throughout pregnancy. 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).

Immunization during lactation

Fifteen mothers were given 0.75 ml of anti-IGF-1 IgG (capable of neutralizing 5.7 µg of IGF-1) *in vitro*, twelve received 0.75 ml of anti-IGF-2 IgG (capable of neutralizing 4.2 µg of IGF-2) and eighteen received a similar volume of IgG purified from normal sheep serum.

Within 24 hours of birth, litter sizes were adjusted to 8; the weights of the pups were recorded every other day throughout the trial. Calculation of milk yield was also made using the formula described by (Sampson and Jansen, 1984).

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

RESULTS

Immunization during pregnancy

There was no difference in the mean maternal weights between the immunized and control animals. The mean birth weight of the pups from the three treatment groups were also similar (Table 1). The postnatal growth rates over the study period were also not significantly different and this was reflected in no significant difference in calculated milk yield between the treated and control rats (Table 1).

TABLE 1: Effects of immunization against IGF-1 or IGF-2 during pregnancy on pup birth weights, postnatal average daily weight gain (ADWG) and milk yields.

	Control	anti-IGF-1	anti-IGF-2
Birth weight (g)	5.97 ± 0.07	5.75 ± 0.05	5.89 ± 0.04
ADWG (g/d)	1.98 ± 0.04	1.86 ± 0.04	2.02 ± 0.02
Milk Yield (g/d)	21.9 ± 1.06	21.5 ± 1.24	20.3 ± 0.87

Immunization during lactation

The mean birth weights of the pups from treated and control dams (Table 2) were not significantly different, and neither was postnatal body weight gain. In addition there was no significant effect of passive immunization of IGFs -1 and -2 in lactating mothers on milk yield as measured by weight gain (Table 2).

TABLE 2: Effects of immunization against IGF-1 or IGF-2 during lactation on postnatal average daily weight gain (ADWG) and milk yields.

	Control	anti-IGF-1	anti-IGF-2
Birth weight (g)	6.01 ± 0.06	6.11 ± 0.11	6.00 ± 0.15
ADWG (g/d)	2.19 ± 0.10	2.02 ± 0.06	2.00 ± 0.16
Milk Yield (g/d)	24.1 ± 1.26	22.2 ± 0.90	18.0 ± 1.97

DISCUSSION

In an earlier report, we hypothesised that retarded postnatal growth in young from mothers immunized against IGF-1 during pregnancy was a result of decreased mammogenesis, decreased galactopoiesis or antibody transfer to the pups (Berry *et al.*, 1993). It has been shown that passive immunization of IGF-1 during the neonatal period does not alter growth in rats (Robinson *et al.*, 1993), so immunization of the pups via transfer of antibody through the milk is unlikely. In the present trials, immunoneutralization of IGF-1 during lactation was also without effect - indicating that a direct effect on galactopoiesis is also unlikely.

A role for IGF-1 in milk production in goats has been suggested by Prosser *et al.* (1990), although others have arrived at different conclusions (Flint *et al.*, 1993). The present study differs from previous studies in that it examines the role of endogenous IGF-1 rather than administration of exogenous IGF-1. This is important since both endogenous and exogenous GH stimulate milk production.

To date, no receptors for GH have been found in mammary tissue, but messenger RNA for GH receptors have been demonstrated (Jammes *et al.*, 1991). The intramammary effect of GH on milk production may be through production of IGFs within the mammary gland (Kleinberg *et al.*, 1990). However, administration of exogenous IGFs or antibodies to the IGFs failed to have any effect, despite antibodies readily reaching the intercellular spaces in mammary tissue (Lascelles *et al.*, 1981).

The lack of effect of neutralization against IGF-2 on birth weight is consistent with our preliminary studies (Spencer *et al.*, 1993). Prosser *et al.* (1994) have recently investigated the effect of exogenous IGF-2 on lactation in goats; they concluded that during the first 6 hours of a unilateral infusion with IGF-2 there was a slight increase in milk yield compared with the non-infused gland (+13 g), but no significant increase compared with saline infused animals (+6g). The results of the present studies do not support the postulate of a role for IGF-2 in lactation; this conclusion is consistent with the findings of Flint *et al.* (1993).

Thus these results indicate that neither circulating, nor probably intramammary IGFs, influence lactation in the rat. Thus it seems likely that the effects of GH on stimulating lactation are mediated via another factor(s).

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