Effects of food restriction on the responses of the mammary gland and adipose tissue to prolactin and growth hormone in the lactating rat

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Abstract

Exogenous GH is used extensively in the USA to stimulate milk production in dairy cattle but its effectiveness is reduced in undernourished animals. It has been proposed that GH increases milk yield by stimulating IGF-I secretion and that this IGF-I-response is nutritionally sensitive and thus acts as a ‘sensor’ of energy balance. To investigate this possibility, we placed lactating rats on three planes of nutrition, ad libitum, 50% or 25% of ad libitum for 48 h. Subgroups of these animals were treated for 48 h with bromocriptine, to suppress prolactin secretion, and anti-rat GH, to neutralize GH action. From 24 to 48 h some of the treated animals were assessed for their milk yield response to prolactin or GH.

Food restriction reduced milk yield in control rats by approximately 50% and was accompanied by a catabolic state, as judged by lipid mobilization from adipose tissue and by low concentrations of serum insulin, IGF-I, triiodothyronine and thyroxine, and increased serum non-esterified fatty acid concentrations. In animals fed ad libitum, anti-rat GH plus bromocriptine treatment produced an 80% decrease in milk yield and a dramatic fall in the activity of acetyl-CoA carboxylase in mammary tissue. GH was able to stimulate milk yield when given from 24 to 48 h; however, its effectiveness decreased progressively as food intake was reduced. The milk yield response to GH was accompanied by an increase in serum IGF-I concentrations and this response also decreased progressively with reduction of food intake, consistent with the hypothesis that IGF-I determines the milk yield response to GH and thus regulates GH action on the mammary gland in a nutritionally dependent fashion. However, the milk yield response to prolactin and the milk yield of control rats decreased in line with food intake without any changes in serum IGF-I concentrations. This clearly indicates that factors other than IGF-I are responsible for restricting milk yield. In order to assess other possible candidates for this role, we monitored serum glucose, non-esterified fatty acids, insulin triiodothyronine and thyroxine concentrations, but found no evidence for any simple relationship between these parameters and the milk yield response to prolactin and GH.

Surprisingly we found that the ability of GH or prolactin to prevent epithelial cell loss in the mammary gland was completely insensitive to nutrient intake, despite the fact that IGF-I is considered to be an important survival factor for mammary epithelial cells.

Finally, we also demonstrated that, at least during short-term food restriction, the lactating rat is capable of mobilizing significant amounts of lipid from adipose tissue, such that it could provide the total output of triglyceride in milk, which is much greater than has previously been proposed.

Introduction

Growth hormone (GH) stimulates milk production during lactation and it has been proposed that this is mediated indirectly via the production of insulin-like growth factor-I (IGF-I) (see Bauman & Vernon 1993). Use of exogenous GH to stimulate milk yield is now common in the USA, and concern has been expressed that this may ‘push’ animals into negative energy balance and run the risk of ‘metabolic collapse’. When nutrition is inadequate, however, the effect of GH on milk output is considerably reduced and, consistent with this, so is the increase in serum IGF-I concentrations (McGuire et al. 1992, 1995). It has thus been proposed that IGF-I serves as an indicator of ‘energy balance’ (McGuire et al. 1995) and thus limits any potentially harmful effects of exogenous GH. Consistent with this hypothesis, serum concentrations of IGF-I are characteristically high when animals are in positive energy balance and low when they are in negative energy balance.

In order to test the hypothesis that IGF-I is a sensor of energy balance for milk production in the lactating rat, we
took advantage of a model we have developed in the lactating rat in which both GH and prolactin (PRL) stimulate milk secretion (Barber et al. 1992, Flint et al. 1992). Since the effects of exogenous GH and PRL are only evident in GH- and PRL-deficient rats, all animals were first treated with anti-rat (r)GH and bromocriptine. Whereas the effects of GH may be mediated via IGF-I, there is no evidence that the metabolic effects of PRL also require IGF-I to serve as a mediator for its actions on the mammary gland. We therefore examined the influence of food restriction on the response to GH and PRL to examine its effects on milk secretion stimulated via IGF-I-dependent and -independent mechanisms.

To assess the metabolic status of the animals we measured several parameters related to adipose tissue metabolism, since animals in negative energy balance have to mobilize lipid from adipose tissue whereas, when in positive energy balance, they store excess energy in adipose tissue. Adipocyte mean cell volume was measured, as this is related to loss or gain of lipid, serum non-esterified fatty acids (NEFAs) were determined as indicators of lipolysis, and acetyl-CoA carboxylase activity was assayed as an indicator of lipogenesis (Vernon 1980). A number of other parameters were also measured as possible signals of energy status, including glucose, insulin and thyroid hormones.

Materials and Methods

Animals

Female Wistar rats, approximately 250 g body weight, were mated and, at parturition, litters were adjusted to 10 pups. All animals were allowed free access to water and food (Labsure irradiated CRM diet, Labsure, Poole, Dorset, UK) except as described below.

Hormones

Recombinant bovine GH (bGH) was a gift from Monsanto (St Louis, MO, USA), and ovine (o)PRL-20 was a gift from NIDDK, Bethesda, MD, USA.

Preparation of sheep anti-rGH serum

Sheep were immunized with rGH and the antiserum was assessed for specificity as previously described (Madon et al. 1986). This antiserum has previously been shown to suppress body weight gain and serum IGF-I concentrations by 90% in rapidly growing rats at the doses used in this study.

Experimental protocol

Lactating rats were treated, commencing on days 12–14 of lactation, with a combination of bromocriptine (Sigma Chemical Co, Poole, Dorset, UK; 500 µg/injection), to suppress PRL secretion, and sheep anti-rGH γ-globulin (150 mg/injection), to neutralize rGH. Treatment was administered twice daily for 2 days by s.c. injection. During these 2 days, one group of the animals (H) were allowed ad libitum access to food; another group had their food intake restricted to 50% of their intake over the previous 24 h (M) and a third group had their food intake restricted to 25% of intake over the previous 24 h (L). During the period 24–48 h, each group was further subdivided into three groups. One received oPRL (500 µg/injection), and another received bGH (500 µg/injection). These doses have previously been shown to completely prevent the decline in milk yield induced by bromocriptine or anti-rGH treatment respectively. A third group received excipient (0.1 M NaHCO₃) at 1000 and 1700 h. An additional three groups of animals served as untreated controls fed at H, M or L levels for the 48 h period. Dam and litter weights were determined daily, and food and water intakes were also recorded daily. Milk yield was determined by the method of Sampson & Jansen (1984). All animals were killed by cervical dislocation 48 h after the start of treatment (i.e. 24 h after receiving PRL or GH replacement therapy). Blood was obtained from the trunk, and serum was prepared by centrifugation at 2000 g for 10 min and stored at −20 °C until analysed for insulin (Vernon et al. 1981), IGF-I (Flint & Gardner 1989), tri-iodothyronine (T₃) and thyroxine (T₄) by RIA (IDS, Boldon, Tyne and Wear, UK) and for serum glucose (Bergmeyer et al. 1974) and NEFAs (Itaya & Ui 1965). The right fourth, fifth and sixth abdominal inguinal mammary glands were removed, weighed and frozen in liquid N₂ for determination of DNA concentration (Labarca & Paigen 1980) and total acetyl-CoA carboxylase activity, i.e. enzyme activity was measured after preincubation with citrate and Mg²⁺ to activate any enzyme in the inactive state (Borland et al. 1994). Parametrial adipose tissue was also removed for determination of mean adipocyte volume and total acetyl-CoA carboxylase activity (Borland et al. 1994). Statistical analyses were performed using ANOVA (general linearized model). Where statistically significant effects of plane of nutrition or hormone treatment were observed, individual group means were compared using Student’s t-test.

Results

Mammary gland metabolism

Milk yield was markedly suppressed by treatment for 48 h with anti-rGH and bromocriptine (Fig. 1a). In ad libitum fed rats, PRL or GH treatment from 24 to 48 h was able to stimulate milk production although not back to control values. This is due to the short treatment period of 24 h since 2–3 days of PRL treatment can normalize milk yield, whereas GH is slightly less effective. The milk yield
achieved in response to PRL or GH also decreased progressively as food intake was reduced, as indeed did the milk yield of the untreated lactating controls. Changes in acetyl-CoA carboxylase activity (thought to be the rate-limiting enzyme for fatty acid synthesis) in mammary tissue paralleled changes in milk yield, with a progressive loss of the ability of GH (P<0.01) or PRL (P<0.01) to increase its concentration as food intake was restricted (Fig. 1b). Mammary DNA content was reduced by approximately 30% after treatment with anti-rGH and bromocriptine (P<0.01). In striking contrast with the metabolic effects, both GH and PRL prevented this loss of DNA, entirely independently of the levels of food intake (Fig. 1a).

Serum IGF-I concentrations

Serum IGF-I concentrations were lower in ad libitum fed lactating rats (31·7 ± 3·2 nmol/l; mean ± s.e.m., n=5) when compared with non-lactating rats (63·6 ± 4·3 nmol/l, n=8; P<0.05). IGF-I concentrations decreased further (P<0.01) when lactating rats were given anti-rGH and bromocriptine, presumably because of the absence of GH activity (Table 1). When GH was administered from 24 to 48 h, it induced a large increase in serum IGF-I concentrations; however, this effect of GH decreased progressively as food intake was restricted (P<0.01). By contrast, PRL did not influence serum IGF-I concentrations significantly.

Adipose tissue metabolism

When food intake was restricted to 50% of ad libitum there were no significant effects on the mean adipocyte volume (Table 2). However, when food intake was restricted to 25% of ad libitum, there were significant decreases (P<0.01) in adipocyte volume, indicative of major lipid mobilization in all three groups of rats that were making significant amounts of milk (i.e. control, PRL- and GH-replacement groups). In the anti-rGH and bromocriptine-treated animals, where milk yield was extremely low, there was no evidence of significant loss of lipid from adipose tissue with the 50% or 25% diets. Changes in the activity of acetyl-CoA carboxylase in adipose tissue were consistent with perceived energy status; thus, in all treatment groups, reducing food intake decreased the activity of this enzyme (Table 2). Conversely, decreasing milk production by endocrine manipulation increased the activity of acetyl-CoA carboxylase in adipose tissue. Thus the endocrine manipulations used in this study produced reciprocal changes in enzyme activity in mammary gland and adipose tissue, whereas restricting food intake decreased activity in both tissues.

Serum hormones and metabolites

Serum insulin concentrations increased 3-fold in anti-rGH- and bromocriptine-treated rats compared with control lactating rats (P<0.01) (Table 1), again consistent with a return to a positive energy balance in these animals. PRL, but not GH, was able partially to prevent this increase (P<0.01). Restriction of food intake led to a progressive decline in serum insulin concentrations (P<0.01) in all but the control lactating rats, where insulin concentrations were already very low. Serum NEFA levels increased in all groups when food intake was restricted to 50% of ad libitum (1·1 ± 0·1 and 1·7 ± 0·1 mmol/l (mean ± s.e.m., n=20) for ad libitum fed and 50% food intake respectively; P<0.01) but did not increase further when food intake was restricted to 25% of ad libitum (1·6 ± 0·2 mmol/l). Serum T₄ levels were reduced in control lactating rats (31 ± 4 nmol/l (mean ± s.e.m., n=5)) compared with non-lactating animals (40 ± 3 nmol/l, n=5, P<0.05 Student’s t-test). T₄ levels were not significantly affected by restricting food intake to 50% of ad libitum but they did decline when intake was restricted to 25% of ad libitum (P<0.02, ANOVA) in control lactating rats (22 ± 1 nmol/l) or rats receiving PRL- (26 ± 3 nmol/l) or GH- (25 ± 1 nmol/l) replacement therapy. By contrast, T₄ levels were increased (P<0.05) in rats given anti-rGH and bromocriptine (41 ± 2 nmol/l), such that they were similar to values in non-lactating rats and were not suppressed even when food intake was reduced to 25% of ad libitum (42 ± 7 nmol/l). Similar effects were evident for T₃, with lactating animals exhibiting lower T₃ levels than non-lactating animals (1·15 ± 0·07 vs 1·42 ± 0·10 nmol/l, P<0.05) and were increased in rats receiving anti-rGH and bromocriptine treatment (1·61 ± 0·2 nmol/l). Serum T₃ concentrations tended to decline with increasing food restriction, although this did not achieve statistical significance, and neither were T₃:T₄ ratios influenced significantly (results not shown). Lactating rats exhibit a modest hypoglycaemia compared with non-lactating animals. Treatment with anti-rGH and bromocriptine, which reduced milk yield, produced a small increase in serum glucose values (P<0.01), whereas PRL- but not GH-replacement therapy produced a small decrease (P<0.05) in serum glucose concentrations. The level of food intake, however, failed to influence serum glucose in any of the groups (results not shown).

Discussion

A number of studies have examined the effects of under-nutrition upon milk production in the rat, although many have included food restriction during pregnancy (see Rasmussen (1992) for review). However, three studies have examined the effects of food restriction solely during lactation (Sainz et al. 1986, Taylor et al. 1986, Grigor et al. 1987), and the magnitude of the effects on milk production were very similar to those reported here. We have extended their findings by demonstrating the ability of rats, at least in the short term, to continue lactating at milk...
Figure 1 Milk yield in ml/day (a), acetyl-CoA carboxylase activity (µmol/min per g tissue) in mammary tissue (b) and DNA content of the mammary gland (c) in control lactating rats, lactating rats treated for 48 h with anti-rGH plus bromocryptine without or with prolactin or GH from 24 to 48 h. Values are means ± S.E.M. for five animals per group. Results were analysed by ANOVA. Where significant differences were found, individual means were tested using Student’s t-test. Values not sharing the same superscript differ (P<0.05).
yields in excess of 40% of normal, even when food intake was reduced to 25% of ad libitum. During severe food restriction, adipocyte mean cell volume fell from 346 pl to 222 pl (i.e. by 36%). Based upon a body fat content of 30 g in our rats (Kanto & Clawson 1980), this would represent a loss of approximately 11 g of lipid over 2 days. Milk yield in our rats was 45 ml/day, with a milk fat concentration of approximately 10% (Flint & Gardner 1994), representing a milk fat output of 9 g in 2 days. Thus the mobilization of adipose tissue could in theory provide all of the triglyceride in milk. This suggests that adipose tissue can make an important contribution to milk yield in rats, quantitatively much greater than proposed by Sadurskis et al. (1991). Clearly, however, such a contribution could not be sustained for longer than 4–5 days at most.

Although we did not conduct true energy balance studies, a number of parameters indicated that a severely catabolic state was induced in all groups that continued to make significant quantities of milk. In particular, there was a substantial loss of adipose tissue, as indicated by the decrease in adipocyte size. Also, body weight was unchanged in ad libitum fed animals (−2 ± 3 g; mean ± s.e.m.) but decreased by 42 ± 3 g and 52 ± 2 g in 50% or 25% ad libitum fed groups respectively (P<0.001 compared with controls). In addition, serum concentrations of NEFA, IGF-I, T_3 and T_4 as well as the suppression of acetyl-CoA carboxylase activity in adipose tissue and the degree of lipid loss from adipose tissue were all consistent with increasing catabolism. Acetyl-CoA carboxylase is considered to be the rate-limiting step in lipogenesis and is acutely responsive to levels of dietary intake (Volpe & Vagelos 1976). In contrast, food restriction did not produce such a severely catabolic state in rats that had their milk production inhibited by treatment with anti-rGH and bromocriptine, since there was no evidence of a major loss of lipid from adipose tissue and serum T_4 concentrations remained elevated. This could be explained in part by the fact that rats treated with anti-rGH and bromocriptine that were given ad libitum access to food, despite reducing their food intake, continued to eat significantly more than non-lactating rats (44 ± 3 g (mean ± s.e.m.) compared with non-lactating values of approximately 20 g) even though they were only producing very small quantities of milk. Even when food intake was restricted to 25% ad libitum, i.e. to approximately 17 g compared with 68 g in lactating animals, this only represents a little less than non-lactating animals would normally eat.

GH plays an important role in milk production in both ruminants (see Bauman & Vernon 1993) and rodents (Flint et al. 1992), although it has been proposed that this effect is indirect, via IGF-I. As serum concentrations of IGF-I generally reflect anabolic status, i.e. high on high planes of...
nutrition and vice versa, IGF-I could serve as a monitor of energy status modulating the response to exogenous GH (McGuire et al. 1992, 1995). We examined this proposed role for IGF-I in a rodent model in which the mammary gland is sensitive to both GH and PRL. By reducing food intake levels we hoped to produce an increasingly catabolic state.

A graded reduction in food intake did lead to a graded reduction in the response to GH in terms of both serum IGF-I concentrations and milk yield. However, there was, if anything, a greater effect of food intake on the milk yield response to PRL, despite unchanged serum IGF-I concentrations. In addition, the decrease in milk production induced by restricting food intake in control lactating rats was also achieved without any change in serum IGF-I concentrations. Although it is possible that nutrient deprivation is sensed in different ways in determining the response to GH and PRL, it seems more likely that they use the same process and that this is not simply via IGF-I.

We cannot entirely rule out the possibility that IGF-I plays some role in maintaining milk production since we have recently shown that GH and PRL may interact through the IGF system to maintain mammary cell survival (Flint & Gardner 1994). Thus we have demonstrated that PRL significantly inhibits the production of IGF-binding protein-5 by the mammary gland, which we believe serves to block IGF-I-mediated mammary cell survival and thus allows apoptosis of the mammary epithelial cells to occur (Tonner et al. 1995). It is interesting, however, to note in the present study that, in animals rendered PRL- and GH-deficient, there was a 30% loss of DNA over 48 h, similar to our previous findings (Flint & Gardner 1994), and that GH or PRL could prevent this completely independently of nutritional intake. Further evidence for a lack of influence of nutrition on mammary epithelial cell survival was demonstrated in the control lactating animals, since food deprivation, although dramatically decreasing milk yield, did not influence mammary DNA content. It thus seems that GH and PRL influence epithelial cell survival and metabolic activity through different pathways, the former being largely independent of energy status with the latter acutely responsive to it. Unravelling these different pathways is one of the major challenges in signal-transduction science and this model may be of value in achieving this goal.

If IGF-I does not serve as an indicator of energy balance status, what does? A number of possibilities exist, including insulin, T₃, T₄, blood glucose concentration (which is known to influence milk yield because of the considerable demand for glucose to synthesize milk lactose), NEFAs (which increase on fasting and which are known to suppress fatty acid synthesis in the mammary gland (Agius & Williamson 1980)) and mammary blood flow (which is also influenced by nutrition). Our data do not support a role for any of these parameters as determinants of the response of the mammary gland to GH and PRL since no simple relationship was found between their concentrations in blood and milk yield response. This does not exclude the possibility that local concentrations of, for example, IGF-I and T₃ could be important, since GH has been shown to increase IGF-I mRNA in the mammary gland (Kleinberg et al. 1990), and the mammary gland also possesses a thyroxine 5'-deiodinase responsible for generating T₂ within mammary tissue (Jack et al. 1994). Tissue sensitivity to IGF-I could also be influenced through nutrition by changes in IGF-I receptors which are present on mammary epithelial cells (Dehoff et al. 1988).

In summary we have shown that lactating rats continue to produce large quantities of milk for at least 48 h despite food restriction. This involves a number of adaptive measures including reduced lipogenic potential and marked lipid mobilization from adipose tissue and a suppression of serum T₄ concentrations. Serum insulin and IGF-I concentrations are already decreased in lactation and, probably as a consequence, these factors did not decrease further upon feed restriction. The responses to GH in terms of milk yield and the increase in serum IGF-I showed a parallel decrease as feed intake was progressively reduced, consistent with the hypothesis that the milk yield response to GH is determined by the IGF-I response. However, the fact that feed restriction also decreased the milk yield response to PRL, and the milk yield of untreated control rats, without any changes in serum IGF-I concentrations, indicates that this hypothesis is probably too simplistic, at least in the rat.

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