Insulin secretion and intestinal peptides during lactation in sheep

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Summary. Intravenous infusions of glucose and/or glucagon-like peptide-1(7–36)-amide (GLP) or somatostatin-28 (S28) were administered to dry and lactating sheep and changes in plasma glucose and serum insulin were followed before, during and after infusion. Basal serum insulin concentrations were significantly lower in lactating sheep but there was no significant difference in plasma glucose concentrations. During glucose infusion, changes in serum insulin concentrations were diminished by comparison with those in dry animals. GLP stimulated insulin secretion during hyperglycaemia in both dry and lactating sheep but, proportionately, the response was significantly greater in the lactating animals. S28 inhibited glucose-stimulated insulin secretion in both dry and lactating sheep and there was no significant difference in the extent of the inhibition between the two physiological states. S28 infusion also inhibited the secretion of GLP from the intestinal tract and this effect was significantly greater in the lactating animals. The results demonstrate a difference in response in the lactating animal to GLP compared with S28. A possible explanation of the difference is a decreased sensitivity (i.e. increased K_m) of the pancreas to the insulinotropic effects of GLP but an increased sensitivity to the inhibitory effects of S28 at tissues other than the pancreas in lactation.

Polypeptides secreted from cells in the intestinal tract in response to nutrient absorption have been shown to augment insulin secretion during hyperglycaemia (Creutzfeldt & Ebert, 1985; Morgan *et al.* 1988; Faulkner, 1991; Fehmann *et al.* 1992). Because of their insulinotropic effects they are commonly known as incretins, but some are also known to have direct anabolic effects on tissues such as adipose (Haji Baba & Buttery, 1991; Oben *et al.* 1991), muscle (Villanueva-Peñacarrillo *et al.* 1994) and liver (Valverde *et al.* 1994). The most effective and frequently studied of these have been glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1(7–36)amide (GLP). More recently, evidence has been accumulating that other peptides, some of which may also be secreted from intestinal cells, counteract the effects of incretins. Somatostatin-28 (S28) and galanin fall into this class (D'Alessio *et al.* 1989; Hsu *et al.* 1991; Fehmann & Habener, 1992; Martin & Faulkner, 1996). Whereas the incretins appear to interact with cell surface receptors linked to G_s proteins which stimulate cAMP production and other intracellular events, galanin and S28 receptors link to the inhibitory G_i proteins (Hsu *et al.* 1991; Fehmann & Habener, 1992) limiting cAMP production.

During peak lactation the endocrine environment maintains the animal in a catabolic state in order to facilitate the partitioning of available nutrients to the mammary gland and milk production and away from deposition of body stores such

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as fat and protein (Vernon *et al.* 1981; Burnol *et al.* 1983). Consistent with this is the characteristic low circulating insulin concentration seen in lactating animals (Vernon *et al.* 1981; Burnol *et al.* 1983). However, lactation is also characterized by an increase in food intake, and this is known to stimulate the secretion of intestinal peptides (Service *et al.* 1983). Recent data indicate that plasma concentrations of GIP and GLP are elevated during lactation in sheep (Faulkner & Martin, 1997) and an increase in total glucagon-like immunoradioactivity has been observed at the onset of lactation in cows (Manns, 1972).

The lactational state, therefore, poses something of a paradox: the physiological conditions require a basically catabolic environment characterized by low circulating insulin concentrations, but the increase in feed intake stimulates increased secretion of insulinotropic, anabolic intestinal polypeptides. One possible explanation of this paradox would be a change in sensitivity to incretins and their antagonists at the pancreatic β-cell level. In the ruminant animal, GLP but not GIP has been shown to be insulinotropic (Faulkner, 1991; Martin & Faulkner, 1996), and S28 antagonizes this effect (Martin & Faulkner, 1996). We have, therefore, followed the effects of GLP and S28 given intravenously to dry and lactating sheep in order to determine any differences in the sensitivity of insulin secretion to these polypeptides in the two physiological conditions.

materials and methods

Animals

Two groups of six Finn–Dorset Horn crossbred sheep 2–5 years old and weighing 50–75 kg were used. All animals were used for experimentation between February and May and six were lactating with two lambs. Lactating sheep were 18–28 d post partum (i.e. at around peak lactation). Dry and lactating animals were given 600 and 1500 g concentrates/d respectively as two meals with hay and water available *ad lib.* as described previously (Martin & Faulkner, 1996). Times of feeding were 08.00 and 16.00. At least 1 d prior to the experiment, two polyvinylchloride catheters were inserted into the jugular veins. Infusions were delivered into one jugular vein and blood samples taken from the other.

Experimental procedures

On each experimental day blood samples for basal values were taken at 15 min intervals between 10.00 and 11.00. At 11.00 the continuous infusion of saline or glucose (25 μmol/kg per min) in sterile water commenced, the glucose infusion being preceded by a bolus injection of glucose (200 μmol/kg). Blood samples were taken at 3 min intervals for 15 min. At 11.15 an additional infusion commenced together with the continuous infusion. This additional infusion consisted of GLP or S28 (both at 1 nmol/kg per min; Sigma Chemical Co. Ltd, Poole BH12 4QH, UK) either alone or with glucose. Blood samples were taken at 3 min intervals for 15 mins. At 11.30 both infusions stopped and blood samples were taken at 3 min intervals for a further 15 mins and then at 15 min intervals till 13·00. Blood samples were processed and glucose, insulin, GIP and GLP were determined as described previously (Martin & Faulkner, 1996). The interassay and intra-assay CV were: for insulin 5·3 and 8·9%, for GIP 6·5 and 11·9% and for GLP 6·9 and 12·2%. The sensitivities of the assays were 21, 22 and 8·2 pM for insulin, GIP and GLP respectively

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Statistical analysis

Statistical significance was determined by ANOVA from analysis of the integrated response as defined by the area under the curve calculated separately for each animal under each infusion condition. Basal values were determined as the means of the values for the first hour of sampling, and these basal values were then subtracted from subsequent measurements (for the period of the infusion and subsequent 15 min) prior to the determination of the area under the curve. Results are given as means ± SE. Some SE for individual treatments are also given in the text for information on individual variation, but were not used for statistical analysis. Differences were considered significant at $P < 0{\cdot}05$.

RESULTS

There were no significant differences in basal plasma glucose concentrations between the two groups ($3{\cdot}38 \pm 0{\cdot}12$ and $3{\cdot}24 \pm 0{\cdot}16$ mM for the dry and lactating sheep respectively), but basal serum insulin concentrations were significantly lower in the lactating animals (157 ± 45 and 50 ± 12 pM for dry and lactating sheep respectively; $P < 0{\cdot}01$). Intravenous infusion of glucose elevated plasma glucose concentrations by $\sim 1{\cdot}4$ mM by the end of the infusion in both groups of animals ($4{\cdot}50 \pm 0{\cdot}24$ and $4{\cdot}78 \pm 0{\cdot}37$ mM for dry and lactating sheep respectively; Fig. 1, Table 1). Serum insulin concentrations rose significantly ($P < 0{\cdot}01$) to 293 ± 72 and 91 ± 14 pM in the dry and lactating animals respectively (Fig. 2). The integrated response of serum insulin to the glucose infusion was significantly ($P < 0{\cdot}01$) diminished in the lactating animals (Table 1).

There was no significant effect on the integrated response of either glucose or insulin when GLP was infused alone (Table 1). Although there did appear to be a rise in serum insulin in both during the actual 15 min of the infusion (Fig. 2), this was not significant. When GLP was infused with glucose, serum insulin concentrations rose to 387 ± 98 and 185 ± 31 pM for the dry and lactating animals respectively (Fig. 2). This rise was significantly different ($P < 0{\cdot}01$) from that with glucose alone. The absolute increases were about the same in both groups (Table 1), but the response relative to that of glucose alone was significantly ($P < 0{\cdot}05$) greater in the lactating sheep (integrated response to glucose being $54{\cdot}6 \pm 8{\cdot}9$% of that to glucose and GLP in lactating sheep, but $70{\cdot}1 \pm 10{\cdot}6$% in dry sheep).

Infusion of S28 alone resulted in a significant ($P < 0{\cdot}005$) increase in the integrated response of plasma glucose in lactating but not dry animals (Table 1). Plasma glucose concentrations increasing to $3{\cdot}52 \pm 0{\cdot}10$ and $3{\cdot}77 \pm 0{\cdot}35$ mM in dry and lactating sheep respectively (Fig. 1). There was no significant change in the integrated response of serum insulin to S28 infusion. When S28 was infused with glucose, serum insulin concentrations decreased to 184 ± 59 and 67 ± 21 pM for the dry and lactating sheep respectively (Fig. 2). The integrated response of insulin was significantly lower than that with glucose alone (Table 1) in both dry and lactating animals. However, neither the absolute nor the proportionate change in the integrated response of insulin to S28 was significantly different between the two groups. As with S28 alone, plasma glucose concentrations tended to rise during S28 with glucose infusion to $4{\cdot}87 \pm 0{\cdot}18$ and $5{\cdot}00 \pm 0{\cdot}42$ mM for dry and lactating animals respectively. The integrated response of glucose was significantly ($P < 0{\cdot}05$) increased in the lactating sheep only (Table 1).

Basal plasma concentrations of GLP were significantly ($P < 0{\cdot}01$) higher in

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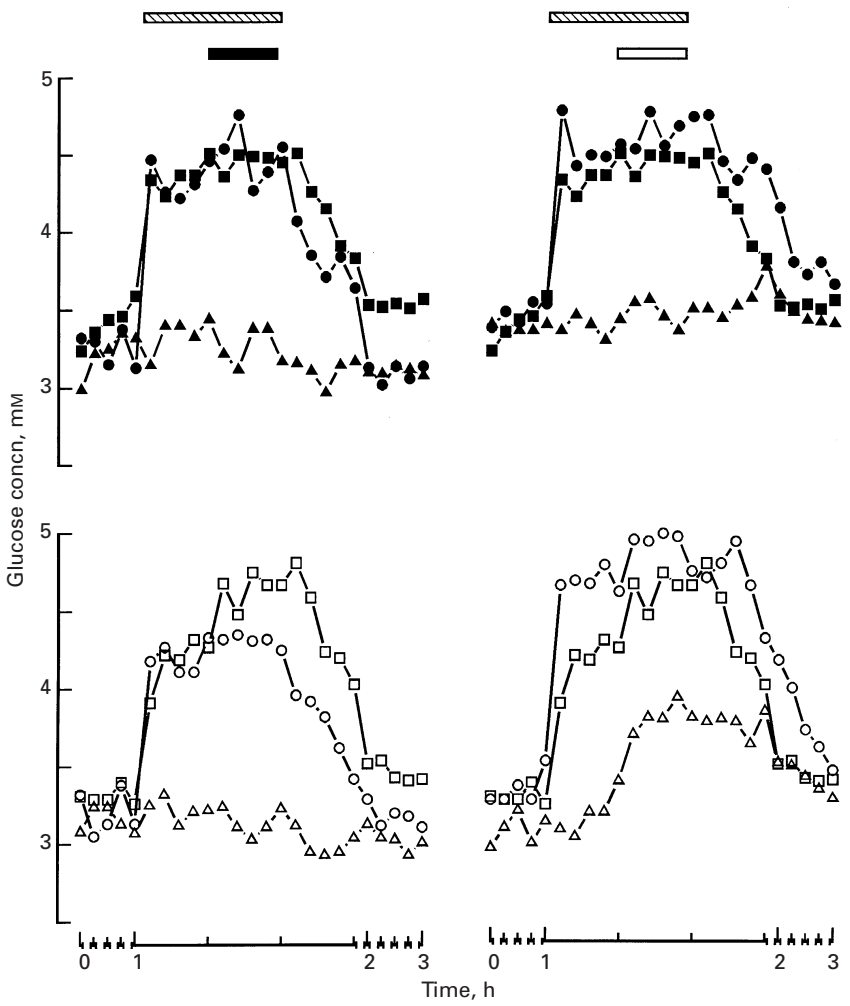


Fig. 1. Changes in the mean concentrations of plasma glucose in dry and lactating sheep during intravenous infusions of glucose and/or glucagon-like peptide-1(7–36)amide (GLP) or somatostatin-28 (S28). Infusions were given as indicated by ▨, glucose; ▬, GLP; ▭, S28. ■, ●, ▲, Dry sheep; □, ○, △, lactating sheep; ■, □, glucose alone; ●, ○, glucose plus GLP or S28; ▲, △, GLP or S28 alone. Glucose was given as a bolus injection of 200 μmol/kg followed by a continuous infusion of 25 μmol/min per kg and GLP or S28 was infused at a rate of 1 nmol/kg per min. Values are means for six animals in each group; for clarity, SE are not shown but are given in the text.

Table 1. *Integrated response of plasma glucose and serum insulin to glucose and/or glucagon-like peptide-1(7–36)amide (GLP) or somatostatin-28 (S28) intravenous infusion in dry and lactating sheep*

Continous infusion	Additional infusion	Glucose, mM per 45 min†		Insulin, mM per 45 min†	
		Dry	Lactating	Dry	Lactating
Glucose	Saline	$59{\cdot}22 \pm 11{\cdot}43^{a}$	$71{\cdot}71 \pm 11{\cdot}01^{a}$	$6{\cdot}47 \pm 1{\cdot}75^{a}$	$2{\cdot}72 \pm 0{\cdot}39^{cf}$
Glucose	GLP	$64{\cdot}29 \pm 12{\cdot}48^{a}$	$58{\cdot}95 \pm 7{\cdot}05^{a}$	$9{\cdot}07 \pm 1{\cdot}62^{b}$	$5{\cdot}66 \pm 1{\cdot}28^{b}$
Glucose	S28	$65{\cdot}85 \pm 9{\cdot}42^{a}$	$93{\cdot}51 \pm 10{\cdot}02^{c}$	$4{\cdot}33 \pm 0{\cdot}97^{c}$	$1{\cdot}90 \pm 0{\cdot}59^{eg}$
Saline	GLP	$0{\cdot}77 \pm 5{\cdot}70^{d}$	$-1{\cdot}83 \pm 4{\cdot}20^{d}$	$1{\cdot}35 \pm 0{\cdot}86^{defg}$	$-0{\cdot}16 \pm 0{\cdot}52^{d}$
Saline	S28	$6{\cdot}03 \pm 4{\cdot}32^{d}$	$31{\cdot}72 \pm 11{\cdot}34^{b}$	$0{\cdot}09 \pm 0{\cdot}74^{deg}$	$-0{\cdot}42 \pm 0{\cdot}26^{d}$

† The SED of the means was 9·88 mM per 45 min for glucose and 1·36 mM per 45 min for insulin.
[a, b, c, d, e, f, g] Values without a common superscript were significantly different: $P < 0{\cdot}05$.

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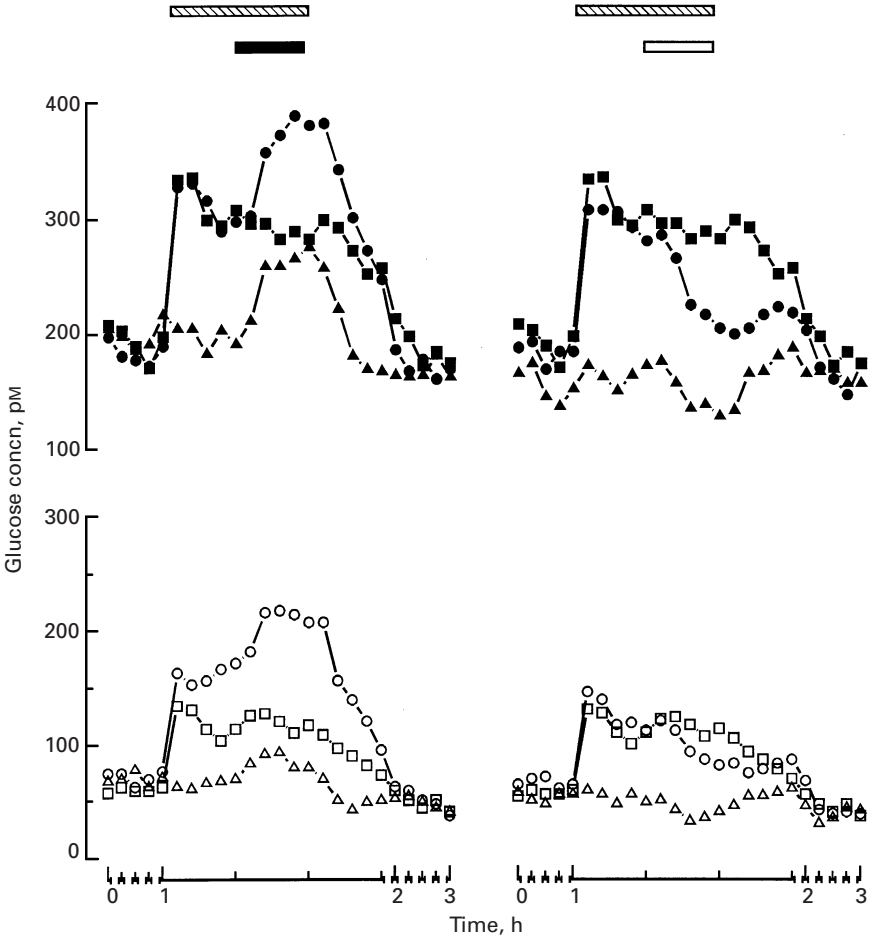


Fig. 2. Changes in the mean concentrations of serum insulin in lactating sheep during intravenous infusions of glucose and/or GLP or S28. Infusions were given as indicated by ▨, glucose; ▬, GLP; ▭, S28. ■, ●, ▲, Dry sheep; □, ○, △, lactating sheep; ■, □, glucose alone; ●, ○, glucose plus GLP or S28; ▲, △, GLP or S28 alone. Glucose was given as a bolus injection of 200 μmol/kg followed by a continuous infusion of 25 μmol/min per kg and GLP or S28 was infused at a rate of 1 nmol/kg per min. Values are means for six animals in each group; for clarity, SE are not shown but are given in the text.

lactating sheep (33 ± 6 and $54 \pm 4{\cdot}5$ pM for dry and lactating animals respectively). Intravenous infusion of GLP increased concentrations to 106 ± 9 and 125 ± 10 pM for dry and lactating animals respectively (Fig. 3). Infusion of S28 resulted in a decrease in plasma GLP concentrations to 23 ± 4 and 32 ± 4 pM for dry and lactating sheep (Fig. 3) and the integrated response of GLP to S28 infusion was significantly ($P < 0{\cdot}004$) greater in lactating compared with dry animals (-357 ± 66 and -848 ± 112 pM per 45 min for dry and lactating sheep respectively). Even allowing for the higher plasma GLP concentrations in lactating sheep, the integrated response relative to basal GLP concentrations was still significantly ($P < 0{\cdot}001$) higher in lactating animals.

Plasma GIP concentrations were also significantly ($P < 0{\cdot}03$) higher in lactating compared with dry sheep (32 ± 10 and 62 ± 10 pM for dry and lactating animals respectively). Infusion of GLP had no significant effect on plasma GIP concen-

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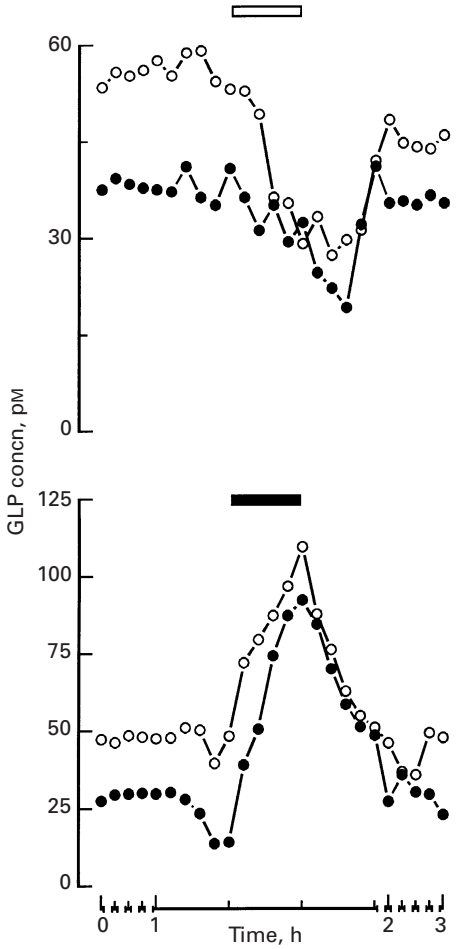


Fig. 3. Changes in the mean concentrations of plasma glucagon-like peptide-1(7–36)amide (GLP) in ●, dry and ○, lactating sheep during intravenous infusion of GLP or somatostatin-28. Infusions were given as indicated by ▬, GLP; ▭, somatostatin-28 infused intravenously at a rate of 1 nmol/min per kg. Values are means for six animals in each group; for clarity, SE are not shown but are given in the text.

trations, but infusion of S28 resulted in a fall in concentrations (18 ± 5 and 32 ± 7 pM for dry and lactating animals respectively). The integrated response of GIP to S28 infusion was not significantly different between the two groups.

DISCUSSION

The results presented in this paper demonstrated an insulinotropic effect of hyperglycaemia in dry and lactating sheep, which was augmented by intravenous GLP. Intravenous infusions of glucose to elevate plasma concentrations by $\sim 1{\cdot}4$ mM in both groups had a significantly greater effect on insulin secretion in dry than in lactating animals. This would indicate a reduced sensitivity to hyperglycaemia at the pancreatic level during lactation. A similar reduced integrated response to glucose injection has been observed in lactating rats (Burnol *et al.* 1983) and dairy cows (Sano *et al.* 1993). Paradoxically, this lowered response to hyperglycaemia in lactation

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occurs at a time when plasma concentrations of the insulinotropic polypeptide, GLP, are significantly elevated in sheep (Faulkner & Martin, 1997). However, administration of exogenous GLP to elevate plasma concentrations some 100% resulted in a similar absolute but greater proportional integrated response of insulin in lactating compared with dry sheep. These observations are consistent with a shift in the shape of the response curve of the pancreas to GLP during lactation such that the concentration of GLP required to give the half maximal response (K_m for activation) in insulin secretion is increased.

Somatostatin has been shown to inhibit insulin secretion through receptor linkage to a G_i protein which antagonizes the effect of GLP (Fehmann & Habener, 1992). Such inhibition has also been demonstrated *in vivo* in humans (D'Alessio *et al*. 1989) and sheep (Martin & Faulkner, 1996). Infusion of S28 with glucose significantly reduced the integrated response of insulin compared with S28 alone. However, neither the absolute nor the proportionate integrated response was significantly different between the two groups. Thus there was no obvious change in the sensitivity of the pancreas to S28 during lactation. Plasma values for S28 during lactation are not available but, as with GIP and GLP, secretion appears to be related to feed intake (Ensinck *et al*. 1989), so that concentrations might be expected to be elevated. Indeed, elevated concentrations of S28 may contribute to the reduced sensitivity of the pancreas to GLP.

When S28 was infused alone, a significant increase in the integrated response of glucose was observed in lactating but not dry animals, a trend which was also evident when S28 was infused with glucose. This could be due to the reduced rate of insulin secretion, but as it was more pronounced in the lactating sheep it is more likely that it represented a change in the rate of non-insulin-dependent glucose release or utilization. GLP is known to stimulate non-insulin-dependent glucose production or utilization by tissues (D'Alessio *et al*. 1995) and this hyperglycaemic effect of S28 may be related to the known inhibition of GLP secretion by S28 (Martin & Faulkner, 1996). This is consistent with the results presented here, which show that S28 inhibits GLP secretion significantly more in lactating compared with dry animals. This indicates that a change in the sensitivity of GLP secretion to plasma S28 concentrations also occurs during lactation.

The phenomenon of changing sensitivity to endocrine stimuli is well established in lactation. The development of insulin resistance in adipose (Vernon *et al*. 1981) and muscle (Vernon *et al*. 1990) tissue has been demonstrated, and serves to maintain an adequate supply of nutrients to the mammary gland. The results reported in this paper show a changed sensitivity of the lactating animal to GLP compared with S28, and may provide some evidence that changes in the sensitivity of the pancreas to GLP occur during lactation, decreasing its efficiency as an insulin secretagogue. In contrast, the sensitivity of the intestinal L-cells to S28 appeared to be changed during lactation, making GLP secretion more sensitive to inhibition. Since it is proposed that GLP stimulates non-insulin-dependent glucose utilization, these changes taken together would reduce the overall consumption of glucose by normal body tissues, thus providing an adequate supply for lactose synthesis and milk production.

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