THE EFFECTS OF SOMATOSTATIN INFUSION ON THE PLASMA PROFILE OF GROWTH HORMONE, INSULIN AND CORTISOL IN SHEEP

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Summary

Four castrated Corriedale sheep were used in an experiment to observe the changes in insulin, growth hormone and cortisol in blood plasma following a prolonged infusion of a high rate of somatostatin (SRIF). The animals were infused with either saline, 25 or 50 μg/kg/h of SRIF for 3 hours. Blood samples were taken every 20 minutes until 1 hour following the end of the SRIF infusion. Both SRIF infusion levels suppressed the release of insulin into plasma to approximately 3.5 mU/l. The SRIF infusions reduced the concentration of growth hormone to barely detectable levels. Following the withdrawal of SRIF there was a massive release of growth hormone. The plasma concentration of growth hormone reached 60 ng/ml within 20 minutes, the length of the growth hormone discharge was in excess of 1 hour. The extent of the discharge of growth hormone following the SRIF infusions was greater than that suppressed by the infusion. The SRIF apparently caused an increase in the plasma concentration of cortisol at the end of the infusion and following its withdrawal. This is possibly associated with some change in the metabolic rate associated with the suppression of insulin or glucagon release. The present experiment demonstrates that a high rate of SRIF infusion can not completely inhibit the release of insulin into the plasma.

(**Key Words** :SRIF, Growth Hormone, Insulin, Cortisol.)

Introduction

The distribtion and physiological roles of somatostatin (or somatotropin release inhibiting factor; SRIF) within the body are very wide and varied. Amongst many other things, exogenous administration is known to inhibit the secretion of insulin, growth hormone and glucagon (Martin 1985). Indeed, the studies of Hafs et al. (1977) and Bergman et al. (1984) in bulls and dogs respectively noted marked reductions in the plasma concentrations of insulin during the infusion of SRIF. However, despite the fact that there are many reports detailing the reduction in insulin levels during SRIF infusion in ruminants, these studies have used relatively low rates of SRIF infusion and have noted far from complete inhibition of insulin release (e.g. Hafs et al., 1977, Brockman and Greer, 1980). As far as we know, there are no reports which indicate that insulin secretion can be completely inhibited by SRIF infusion in the ruminant. As part of a wider programme to determine insulin-mediated and non-insulin-mediated glucose uptake in sheep, information was required concerning the extent of the maximum possible suppression of insulin release by SRIF. Consequently, the primary aim of the present experiment was to observe the plasma profiles of insulin following a prolonged infusion of SRIF at a high rate. SRIF is also known to powerfully blockade the release of growth hormone, but that this inhibition is only maintained in the presence of SRIF, such that there is a rebound release of growth hormone following it's removal (Robinson and Clark, 1989). Thus, a secondary objective of this study was to observe the release of growth hormone during and following the administration of SRIF.

Materials and Methods

Four castrated male Corriedale sheep (9 months of age) were used. They were kept in metabolic crates under continuous lighting and at a constant 22℃. The average weight of the sheep was 24.8 kg (SE = 0.19). Each sheep was fed 800 g per day of lucerne hay cubes (DM = 85.7%, GE = 15.8 MJ/kg, CP = 15.3%, OM = 76.3%)

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in two equal meals at 08:30 and 16:00 each day. Water and salt blocks were available *ad libitum*. The sheep were allowed to adjust to these feeding and environmental conditions for 21 days before the experiments were performed. On the day prior to each experimental day polyvinyl catheters were inserted into both jugular veins of the animals. Catheter patency was maintained by flushing with a 1,000 units per ml solution of heparin. The catheter on the left side of the animal was used for all infusions whilst that on the right was used for blood sampling.

On the first experimental day at 11:00 hours (2.5 hours after feeding) 25 μg/kg/h of synthetic SRIF 14 (SRIF-25) was infused for 3 hours. On the following experimental day the animals were infused with saline and on the final day with an infusion of 50 μg/kg/h of SRIF (SRIF-50). The SRIF was obtained from Peptide Institute, Inc., USA. At least 7 days separated the experiments. The two rates of SRIF infusion were chosen to ensure that a maximal suppression of insulin occurred. Blood samples (10 ml) were taken 20 minutes prior to and immediately before the start of the infusions. Further blood samples were then taken at 20 minutes intervals from the start of the infusion until 240 minutes, that is until one hour after the infusion had stopped. The blood samples were put into heparinised containers and placed on ice. The blood samples were stored as plasma at −20°C until they were assayed for growth hormone (Johke, 1978), insulin (Eiken RIA kit, Eiken Chemical Co., LTD, Japan) and cortisol (Eiken RIA kit).

The profiles of the hormones were split into three periods: prior to, during and after the SRIF or saline infusions. The area under the profiles in the three periods were calculated assuming linearity between the points. All values presented are the means (with standard errors) of four animals. Comparisons between treatments were made using paired Student's t-tests.

Results

The plasma concentration of insulin was considerably reduced by both levels of SRIF infusion. However, the circulating concentrations of insulin were not completely inhibited and they quickly returned to the values observed following the saline infusion, once the SRIF was removed (table 1). The reduction in insulin concentrations during the SRIF infusion was of the order of 75%.

There was a significant and marked reduction in control insulin levels over the course of the experiment, the reduction being in excess of 50 percent (figure 1). There was also an apparent reduction in the basal concentration of insulin over the course of the SRIF

TABLE 1. AREA UNDER CURVES OF INSULIN, GROWTH HORMONE AND CORTISOL: BEFORE DURING AND IMMEDIATELY AFTER THE SALINE OR SOMATOSTATIN INFUSIONS

	Somatostatin Infusion Level (μg/kg/h)			Significance of Difference	
	0	25	50	0 v 25 μg/kg/h (SED)	0 v 50 μg/kg/h (SED)
Insulin (mU · min/l)					
Before	580.8	409.8	465.0	ns (70.7)	ns (55.3)
Somatostatin	2,049.3	598.8	583.8	*** (77.9)	*** (76.1)
After	700.8	629.0	454.8	ns (168.0)	ns (221.8)
Growth Hormone (μg · min/l)					
Before	64.8	73.3	34.0	ns (23.1)	ns (23.2)
Somatostatin	826.0	211.5	109.3	** (106.5)	** (89.7)
After	287.0	1,391.8	1,617.3	** (234.0)	+ (543.8)
Cortisol (μg · min/l)					
Before	28.6	33.8	25.8	ns (6.4)	ns (5.7)
Somatostatin	217.4	368.1	236.5	ns (84.6)	ns (38.6)
After	64.9	215.3	153.2	** (32.0)	** (10.8)

ns : No significant difference between the saline and respective somatostatin infusion,
+ : Difference tended to significance ($0.05 < p < 0.1$), ** : $0.001 < p < 0.01$,
*** : $p < 0.001$. SED : Standard Error of the Difference.

infusions. This is because during the initial period, insulin levels were significantly greater than those observed immediately following the SRIF infusions. This reduction in baseline insulin values over time on all 3 experimental days is probably associated with feeding, which occurred 2.5 hours prior to the start of the experiment.

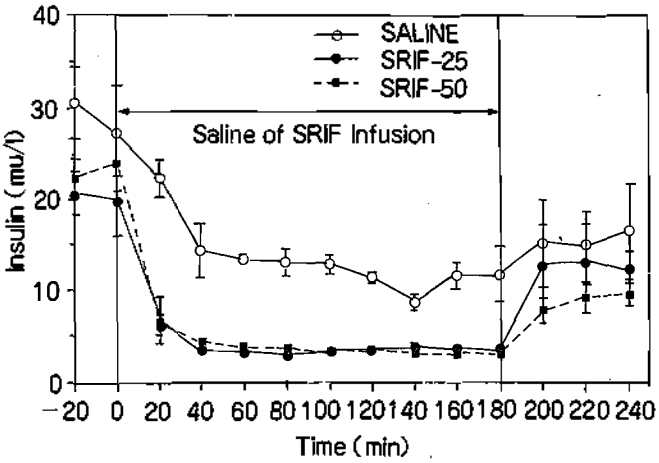

Figure 1. Profile of insulin prior to, during and following the infusion of 0 (saline), 25 (SRIF-25) or 50 μg/kg/hour (SRIF-50) of somatostatin for 3 hours.

The effect of both of the SRIF infusion levels was to clearly reduce the plasma concentrations of growth hormone to levels which were close to and below the detection limit of the assay (figure 2; table 1). This suppression of growth hormone levels remained in effect for the whole of the 3 hours for the SRIF-50 infusion, though the levels of growth hormone tended to increase in three of the four animals used towards the end of the SRIF-25 infusion period. Following the withdrawal of the SRIF there was a profound increase in the plasma levels of growth hormone, reaching levels in excess of 50 ng/ml within 20 minutes. Furthermore, the area under the curve of growth hormone but above the saline infusion values, after the SRIF infusion (area A on figure 2), was greater than the area of the depression of growth hormone concentrations during the somatotropin infusion (area B on figure 2). This effect was significant for the SRIF-25 infusion (1,104.8 vs 614.5 ng·min/ml (SED = 145.02, $p < 0.05$) for areas A and B respectively) and tended towards significance for the SRIF-50 infusion (1,330.3 vs 716.8 ng·min/ml (SED = 273.30, $0.05 < p < 0.1$) respectively). This is despite the fact that the plasma concentration of growth hormone had not returned to basal levels when the final blood sample had been taken, and so the size of area A is an underestimate.

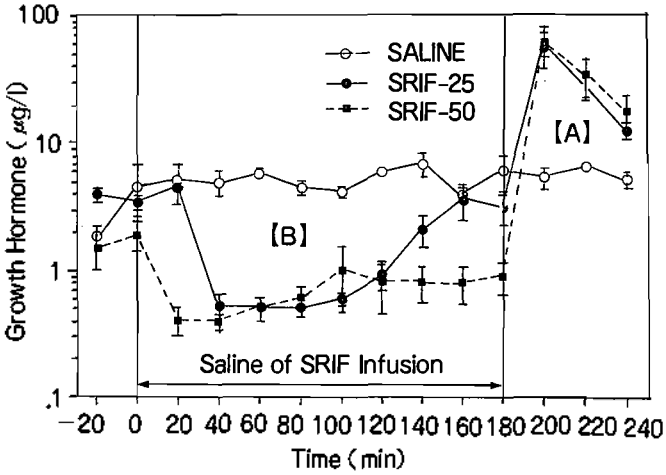

Figure 2. Profile of growth hormone prior to, during and following the infusion of 0 (saline), 25 (SRIF-25) or 50 μg/kg/hour (SRIF-50) of somatostatin for 3 hours.

The area under the profiles of cortisol prior to and during the SRIF infusions were not significantly different to those of the control profile (table 1). However, the profiles of cortisol tended to increase during the course of both of the SRIF infusions, such that individual values in the last hour of both SRIF infusion levels were significantly greater than the respective saline infusion values (figure 3; table 1). The area under the profiles of cortisol following the SRIF infusions were significantly greater than the respective values observed following the saline infusion.

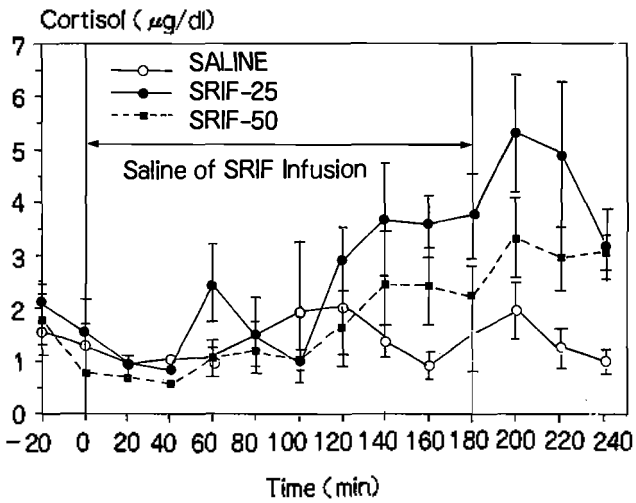

Figure 3. Profile of cortisol prior to, during and following the infusion of 0 (saline), 25 (SRIF-25) or 50 μg/kg/hour (SRIF-50) of somatostatin for 3 hours.

Discussion

It is well established that SRIF inhibits the basal and stimulated rates of secretion of both insulin and glucagon (Martin, 1985) as demonstrated for insulin in the present experiment. However, the present study has also shown that SRIF is not able to completely inhibit the secretion of insulin into plasma. The insulin concentrations observed were similar during both infusion rates, suggesting that for insulin secretion, a maximal effect was achieved. The actual concentrations achieved during the infusions (between 3 and 4 μU/ml) were similar to those observed by Bergman et al. (1984) in dogs, who used a similar rate of infusion to the SRIF-50 rate used in the present experiment.

It is well established that the effect of SRIF is to inhibit the release of growth hormone from the anterior pituitary gland (Davis and Anfinson, 1975). Furthermore, as in the present experiment, a number of reports noted that immediately following the end of SRIF infusions *in vivo* (Hafs et al., 1977) and *in vitro* (Stachura et al., 1988) there is a burst of growth hormone release. SRIF is thought to prevent the secretion of growth hormone without preventing its accumulation in pituitary somatotrophs; when SRIF is removed the accumulated growth hormone is discharged. In the present experiment the extent of the rebound growth hormone release (the peak value was over 10 times the value observed in the saline infusion) was proportionally much greater than that seen in dogs (Cowan et al., 1984; 4.8 times the saline infused level) or bulls (Hafs et al., 1977; 1.8 times the basal level). The greater rebound of the present experiment is likely to be due to a combination of the greater rate of SRIF infusion used as well as the longer period of infusion; Stachura et al. (1988), using *in vitro* rat anterior pituitary incubations, noted that increasing the value of either of these variables increased the size of the growth hormone release. The peak of growth hormone observed in the present experiment was not typical of pulses of growth hormone observed in the natural state in ruminants. In the present experiment growth hormone levels were above basal values for over 1 hour, whereas typically a growth hormone pulse lasts only 30 minutes in the dairy cow (Vasilatos and Wangness, 1981). Again, this difference is probably due to the unphysiologically high dose of SRIF used.

Another result of the present experiment was the fact that the rebound in growth hormone secretion was significantly greater than the depression in growth hormone release caused by the SRIF. This result confirms the *in vitro* observation of Stachura et al. (1988), who noted that SRIF withdrawal combined with GRF resulted in a greatly increased growth hormone discharge; much greater in extent than the suppression of growth hormone release due to the SRIF alone. This result was significant in the present experiment despite the fact that the concentration of growth hormone had not returned to basal levels when the experiment had finished, and so the full area under the profile could not be measured.

The SRIF infusion caused a clear increase in the plasma concentration of cortisol towards the end of the infusion, and following its withdrawal, particularly at the SRIF-25 rate. This result was not apparent in the study of Hafs et al. (1977), though this may be related to the lower infusion rate used in that study and the shorter period of infusion. Presumably, plasma cortisol levels in the present experiment reflect the release of ACTH by the anterior pituitary gland (Zavy et al., 1988). The increase in the concentration of cortisol possibly reflects an increase in the metabolic rate following the depression in the concentration of insulin, though cortisol levels are also known to increase during stress (Zavy et al., 1988).

In conclusion, the present experiment has demonstrated that large infusions of SRIF are not able to completely inhibit the release of insulin from the pancreas such that the plasma concentration of insulin is reduced to zero. SRIF almost completely inhibited the release of growth hormone, though following the removal of SRIF there was a massive rebound in growth hormone secretion. Also, at the end of and following prolonged SRIF infusion, there appeared to be a sustained and significant release of cortisol, which may be related to some change in the metabolic rate.

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Literature Cited

Bergman, R. N., M. Ader, D. T. Finegood and G. Pacini. 1984. Extrapancreatic effect of somatostatin infusion to increase glucose clearance. Am. J. Physiol., 247, E370-E379.

Brockman, R. P. and C. Greer. 1980. Effects of somatostatin and glucagon on the utilisation of [2-^{14}C] propionate in glucose production *in vivo* in sheep. Aust. J. Biol. Sci., 33, 457-464.

Cowan, J. S., P. Gaul, B. C. Moor and J. Kraicer. 1984. Secretory bursts of growth hormone secretion in the

dog may be initiated by somatostatin withdrawal. Can. J. Physiol. Pharmacol., 62:199-207.

Davis, S. L. and M. Anfinson. 1975. Dose response influence of prostaglandin E_1 and somatostatin on plasma levels of growth hormone, prolactin and thyrotropin in sheep. J. Anim. Sci., 41:173-177.

Hafs, H. D., T. E. Kiser, N. B. Haynes, J. S. Kesner and J. N. Stelflung. 1977. Release of pituitary hormones, cortisol, testosterone and insulin in response to prostaglandin F2α given during intra-carotid infusion of somatostatin in bulls. J. Anim. Sci. 44:1061-1066.

Johke, T. 1978. Effects of TRH on circulating growth hormone, prolactin and triiodothironine levels in the bovine. Endocrinol. Japon. 25:19-26.

Martin, C. R. 1985. Endocrine Physiology. Oxford University Press, New York.

Robinson, I. C. and R. G. Clark. 1989. Growth promotion by growth hormone and insulin like growth factor-1 in the rat. In, Biotechnology in Growth Regulation. Editors, Heap, R. B. Prosser, C. G. and Lamming, G. E. Butterworths, London.

Stachura, M. E., J. M. Tyler and P. K. Farmer. 1988. Combined effects of human growth hormone (GH) releasing factor 44 (GRF) and somatostatin (SRIF) on post-SRIF rebound release of GH and prolactin: A model for GRF-SRIF modulation of secretion. Endocrinol., 123:1476-1482.

Vasilatos, R. and J. Wangness. 1981. Diurnal variations in plasma insulin and growth hormone associated with two stages of lactation in high producing dairy cows. Endocrinol., 108:300-304.

Zavy, M. T., W. A. Phillips, P. E. Juniewtz and D. L. Vontungeln. 1988. Effect of genotype on basal and ACTH stimulated cortisol response in beef steers during weaning and transit stress. J. Anim. Sci., 66 (Suppl 1), 234-235.