Effect of leukemia inhibitory factor [LIF] on feed intake and body temperature in sheep

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Abstract

Leukemia inhibitory factor (LIF) has been suggested to function as a potent inhibitor of feed intake in rodents. These studies were designed to determine whether LIF was found in the ovine hypothalamus and whether LIF inhibited feed intake in sheep. Sheep hypothalami were used to clone LIF to indicate presence of the gene in the hypothalamus. The sequence was similar to published data. Another group of sheep were provided intra-ventricular (ICV) cannulas and injected with doses of LIF at 250, 500, 1000 and 2500 ng per sheep, ICV. Feed intake was inhibited by the 1000 and 2500 ng dose (trt, P<0.0001; time X trt, P<0.02). All doses of LIF elevated temperature above 40 C, indicating a fever. In a second experiment, the sheep were injected ICV with 2500 ng LIF, and blood samples collected at 10 min intervals for 6 h for assay of luteinizing hormone (LH), growth hormone (GH) and 30 min interval samples assayed for glucose and free fatty acids.There was no effect of LIF on GH. There was no effect of trt for LH, but there was a time X trt interaction indicating reduced LH (P<0.0001). There was an effect of trt and time X trt interaction indicating elevated plasma free fatty acids (P<0.03; 0.001) and glucose (P<0.006; 0.0001). The effects of LIF on feed intake and other parameters is similar to the effects of LPS and leads to a hypothesis that LIF expression in response to LPS may be a component of the mechanism for feed intake inhibition in disease. While the effects of LIF to increase glucose and free fatty acids are similar to those seen with LPS in sheep, they are opposite to the effects of LIF in rodent models.

Introduction

The metabolic response to fasting is a decrease in metabolic rate and an increase in mobilization of fats. The adaptations in metabolism and shifts in energy utilization are made to allow the animal to survive until food becomes available. In contrast, disease is a catabolic process that produces an increase in production of cyto-kines and subsequent intracellular modifiers that result in specific alterations in metabolism. Thus changes in the animal from anabolic to catabolic metabolism occur, characterized by an increased metabolic rate and sub-sequent mobilization of fats for energy. If the disease continues, the animal can become cachectic. Here there is no food intake, metabolic rate is enhanced to fight the disease process and the animal will begin to mobilize proteins as well as fats. The hallmark of cachexia is a progressive body mass wasting (Sartin et al., 2005).

A study in laboratory animals (Plata-Salaman, 1996) has suggested that IL-6 subfamily members may regulate feed intake inhibition in rodents. Indeed, leukemia inhibitory factor (LIF) was found to be a potent appetite suppressor. In other studies, LIF gene therapy, introduced into the lateral ventricle of the brain, produced a dose related reduction in feeding and body weight in rats. Moreover, serum leptin, insulin and free fatty acids were reduced and glucose unchanged by the constant overexpression of LIF within the brain (Beretta et al., 2002). In addition, LIF was found to stimulate POMC gene expression in the pituitary (Guignat and Bertherat, 1999). Since one product of the POMC gene in the hypothalamus is the appetite inhibitory neurotransmitter, a-melanocyte stimulating hormone (a-MSH), it suggests that LIF may play a role in the disease associated reduc-tion of feed intake observed following endotoxin or cytokine administration. Indeed, a recent paper (Grossberg et al., 2010), indicates an increase in gene expression for LIF in the hypothalamus and provides data to suggest LIF may mediate interleukin-1 stimulated inhibition of feed intake in laboratory animals. Therefore, this study was initiated to determine if LIF injection in sheep would replicate selected physiological changes observed following endotoxin in sheep (elevated body temperature, plasma glucose, plasma free fatty acids, plasma growth hormone and reduced feed intake and plasma luteinizing hormone; Sartin et al., 1999; 2005; Daniel et al., 2002).

Methods

Experiment 1. **Effects of LIF on feed intake**. This study sought to determine whether LIF injection ICV would provide a dose related decrease in feed intake and elevated body temperatures. Five wethers were placed on raised floor pens and treated with antibiotics and dewormed prior to being provided ICV catheters connected to a Perf™ (Ommaya Style) reservoir *(Vygon Neuro, Valley Forge, PA)*. Sheep were provided feed and water *ad libitum*. Sheep were injected via ICV cannula with saline or LIF (250, 500, 1000, 2500 ng/ sheep; *Santa Cruz Biotechnology*). Feed intake was measured by taking weights of feed remaining (from a known amount) at 2, 4, 6, 8, 10, 24 and 48 h after treatments. After each feed measurement, fresh feed was weighed and offered to the sheep. At each feed measurement, a rectal body temperature was recorded. Sheep were randomly provided each treatment (5 treatments randomly given to each sheep so at least each treatment was provided each day) with a one week interval between treatments.

Experiment 2. **Effects of LIF on key hormones and metabolites**. This study sought to determine whether LIF mimics the effects of endotoxin to increase growth hormone concentrations, reduce luteinizing hormone concentrations, and elevate glucose and free fatty acids in plasma. Sheep (n=6) with ICV and jugular cannulas were used in these experiments. On the day of an experiment, a blood sample is taken at -10 min, 0 min, the sheep injected via the ICV cannula with either saline or LIF (2500 ng; the dose of causing a maximal inhibition of feed intake from *experiment 1*), and blood samples collected at 10 min intervals for 5 h. Samples were stored frozen for later assay of LH and GH by validated radioimmunoassay and for free fatty acids and glucose by spectrophotometric assays. Pulse patterns of LH were determined using Cluster analysis.

Statistics. **Cumulative food intake and body temperature are analyzed** using a univariate split-plot model approach for repeated measures with JMP software *(SAS Institute, Cary, NC)*. Effect of LIF on circulating concen-trations of growth hormone, glucose, and free fatty acids were analyzed using the univariate split-plot method for repeated measures analysis with JMP Software *(JMP ver. 6, SAS Institute Inc., Cary, NC)*. Means separation were per-formed with Tukey's HSD. Mean concentration of LH, area under the curve, peak number, average peak height and nadir following LIF administration were determined using CLUSTER pulse analysis procedures *(Veldhuis and Johnson, 1986)*. Mean concentration of LH, area under the curve, peak number, average peak height and nadir were then analyzed effect of LIF treatment, date and id as a Latin Square Design using JMP Software.

Results

Figure 1:
Effect of ICV injection of LIF on body temperature in sheep.
Temperatures above 40° C were considered a fever. n= 5 per treatment.

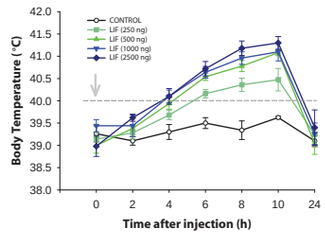

Figure 2:
Effect of ICV injection of LIF on feed intake in sheep.
There was an effect of treatment, P<0.0001. When the saline, 1000 and 2500 ng doses of LIF were used in the analysis, there was an effect of treatment, P<0.0001; Time, P<0.0001; and a time by treatment interaction, P<0.02. Significant differences from saline controls are indicated by * at P<0.05. n= 5 per treatment.

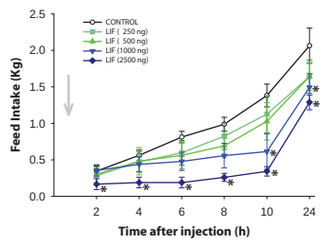

Figure 3:
Effect of ICV injection of LIF on plasma growth hormone concentrations in sheep.
Samples were collected at 10 minute intervals. There was no effect of treatment, P<0.09; and an effect of time, P<0.03; and no treatment by time interaction, P<0.32. n= 5 per treatment.

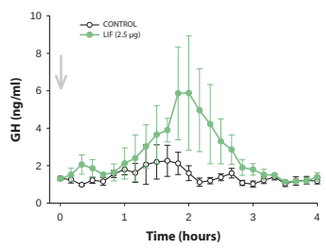

Figure 4:
Effect of ICV injection of LIF on plasma luteinizing hormone concentrations in sheep.
Samples were collected at 10 minute intervals. There was no effect of treatment, P<0.08; an effect of time, P<0.03; and a treatment by time interaction (P < 0.0001).
* differs from saline, P<0.05; + differs from pre-injection control, P<0.05. n= 5 per treatment.

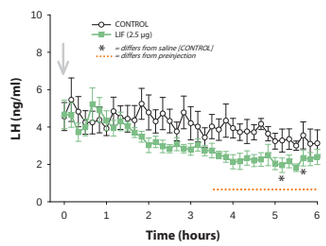

Figure 5:
Effect of ICV injection of LIF on plasma glucose concentrations in sheep.
There was a significant effect of treatment, P<0.006; an effect of time, P<0.0001; and a time by treatment interaction, P<0.0001.
* differs from saline, P<0.05. n= 5 per treatment.

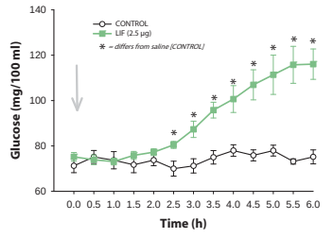

Figure 6:
Effect of ICV injection of LIF on plasma free fatty acid concentrations in sheep. There was an effect of treatment, P<0.03; effect of time, P<0.001; and a time by treatment interaction, P<0.001.
For LS mean differences, * differs from saline, P<0.05. n= 5 per treatment.

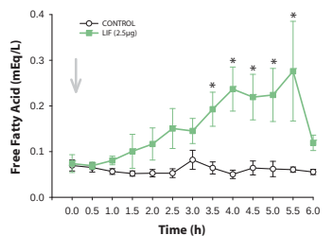

Figure 7:
Partial sequence of ovine leukemia inhibitory factor, cloned from sheep hypothalamus and compared to the bovine sequence (Blast search results).

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Query 1     CAGCAGAATGACCACCATCAGTGCCTTGGCTGACCTTAAACGGTCTGGTTGGAGCTCAG   59
Sbjct 1129  CAGCAGAATGACCACCATCAGTGCCTTGGCTGACCTTGAAA-GGTCTGGTTGGAGCTCAG 1187

Query 60    GCAGCCCAGAGGGGCTGGGATCCGGAAGGACCCTTGGCTCCTACAGGTATGGCGAGTT--  117
Sbjct 1188  GCAGCCTGGAGGGGCTGGGATCCGGAAGGACCCTTGGCTCCTACAGGTATGGCGAGTTGG 1247

Query 118   ---G----GAACGGGAGCTGTGGTTTGAGATGCTGCCTTACTTGGGTGAGACTGGGGAGTT 171
Sbjct 1248  AAGTCTAGAACGGGAGCTGTGGTTTGAGATGCTGCCTTGCTTGGGCAAGACTGGGGAGTT 1307

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Query 232   CGGGAAGGCAGAGAGAGGCCACATGCAGGCCGGGGAAGGAAGAGAGGCCCTGGAAAGGCT  291
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Table 1:
Effect of leukemia inhibitory factor (LIF) on plasma luteinizing hormone (LH) pulse parameters.

	Mean AUC	Peaks (#)	Peak (ht.)	Peak	Na^{dir}
Saline (n=5)	4.7+.25 1	404.8+150.0	4.4+0.8	5.5+0.2	3.5+0.1
LIF (n=5)	3.3+.25	1059.7+114.6	2.5+0.6	4.3+0.2	2.7+0.1
P-value (S VS LIF)	0.08	0.3	0.3	0.06	0.05

Conclusions

The data indicates that intracerebroventricular injection of leukemia inhibitory factor inhibits feed intake in sheep. It also has similar effects to endotoxin treatment on body temperature and on plasma glucose, and free fatty acid concentrations.

The inhibition of plasma luteinizing hormone concentrations is less pronounced but similar to the effects of endotoxin. While not statistically significant, the growth hormone concen-trations mirror changes seen with endotoxin in sheep. Perhaps higher concentrations of leukemia inhibitory factor would provide better results with the plasma hormones.

The effects of leukemia inhibitory factor on glucose and free fatty acids is the opposite to the effects of chronic delivery via gene therapy, but there is a species difference as well as a long term versus short term administration difference.

In addition, leukemia inhibitory factor is present in the ovine hypothalamus. We speculate that leukemia inhibitory factor is a physiological component of the response to endotoxin adminis-tration, but further experimentation is required to prove this hypothesis.

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