

EFFECT OF INSULIN ON FEVER IN ENDOTOXIC SHEEP

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Abstract

A study was conducted to determine the effect of intravenous (i.v.) administration of insulin on fever in sheep challenged with bacterial endotoxin, lipopolysaccharide (LPS). Six castrated male Suffolk-cross wethers were randomly assigned to receive one of the following treatment combinations i.v: Saline control (SAL+SAL); SAL + LPS (0.06 µg kg⁻¹ BW) or various doses of insulin (I) (2, 6, 12 or 20 mU kg⁻¹ BW) + LPS (0.06 µg kg⁻¹ BW). Serial blood samples were collected at hourly intervals for 10 h after the start of i.v injections. Glucose concentrations in the plasma were measured. Rectal temperature was monitored at the same time as for serial blood sampling. Temperatures for the saline control sheep (SAL+SAL) remained relatively constant throughout the study period ranging from 38.9 ± 0.1 to 39.1 ± 0.1 °C. The SAL+LPS treated sheep had significantly (P<0.05) elevated temperatures compared to the saline controls from 1 to 8 h post LPS injection. The sheep injected with 12mUI +LPS had significantly (P<0.05) lower body temperature compared to the SAL +LPS treated sheep from 3 to 6 h post LPS injection. Within the insulin + LPS treatment combinations the 12mUI +LPS combination was found to significantly reduce (P<0.05) body temperature in sheep to levels similar to the saline controls from 5 to 8 h after LPS injection. Sheep on the SAL+LPS or I+LPS treatments had reduced (P<0.05) glucose levels than the saline control sheep from 5 to 8 h post LPS injection. This study demonstrates the ability of insulin to reduce fever in LPS challenged sheep.

Key words: Endotoxin, fever, glucose, insulin, sheep

EFFET DE L'INSULINE SUR LA FIÈVRE DANS LES MOUTONS INFECTÉS ENDOTOXINE BACTÉRIENNE

Résumé

Une étude a été menée afin de déterminer l'effet de l'administration par voie intraveineuse (iv) de l'insuline sur la fièvre des moutons infectés d'endotoxine bactérienne lipopolysaccharide (LPS). Six mâles croisés Suffolk castrés ont été randomisés pour recevoir l'une des combinaisons suivantes de traitement IV: Saline de contrôle (SAL + SAL); SAL + LPS (0,06 mg kg⁻¹ pc) ou différentes doses d'insuline (I) (2, 6, 12 ou 20 kg⁻¹ mU BW) + LPS (0,06 mg kg⁻¹ pc). Des échantillons de sang ont été collectés en série à une cadence horaire de 10 h après le début des injections. Les concentrations de glucose dans le plasma ont été mesurées. La température rectale a été contrôlée au même moment que le prélèvement sanguin. Les températures pour les moutons témoins (SAL + SAL) sont demeurées relativement constante pendant toute la période d'étude allant de 38,9 ± 0,1 à 39,1 ± 0,1 °C. Chez les moutons traités SAL + LPS elles ont significativement (P < 0,05) augmentées par rapport aux contrôles salines entre 1 à 8 heures après l'injection. Le mouton injecté avec 12mUI + LPS avaient une température corporelle significativement (P < 0,05) plus basse que celle des animaux SAL + LPS 3 à 6 h après l'injection de LPS. Au sein des groupes traités à différentes concentration d'insuline ceux ayant reçu LPS l'12mUI ont eu une température corporelle considérablement (P < 0,05) abaissée pour atteindre celle du groupe 5 à 8 h après l'injection. Cette étude démontre la capacité de l'insuline à réduire la fièvre chez les moutons infectés d'endotoxines bactérienne lipopolysaccharide

Mots clés: endotoxine, la fièvre, de glucose, d'insuline, les moutons

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Introduction

Fever, anorexia, and reduced physical activity in ruminants are characteristic features of the coordinated host response to lipopolysaccharide (LPS) challenge, microbial infection or chronic inflammatory diseases which are generally thought to be mediated by host-derived proinflammatory cytokines acting on the central nervous system (Baile *et al.*, 1988; Plata-Salaman, 1991). Experimental studies in humans, mice and rats indicate that insulin can act as an anti-inflammatory agent by decreasing the proinflammatory response and increasing the anti-inflammatory cascade (Jeschke *et al.*, 2002; Dandona *et al.*, 2009). Insulin treatment was reported to dampen inflammatory and acute phase responses by decreasing interleukin-6 (IL-6) and acute phase proteins in humans (Jeschke *et al.*, 2010). Also the administration of insulin subcutaneously was reported to decrease proinflammatory cytokine expression in the liver and serum levels of tumor necrosis factor- α (TNF- α) interleukin-1 β (IL-1 β) and IL-6 in endotoxic rats (Jeschke *et al.*, 2004). Differences exist among species with regard to their endotoxin sensitivity (McKusky *et al.*, 1984). Moreover, reports on the effects of insulin on the inflammatory response and metabolic changes in endotoxic sheep is limited.

The purpose of this study was therefore to determine the ability of insulin to suppress fever in sheep challenged with LPS. The effect of LPS on carbohydrate metabolism was also characterized by determining the levels of glucose in the plasma.

Materials and Methods

Experimental procedures

Six castrated male Suffolk-cross wethers (weight range of 58.6 to 65.9 kg) were housed indoors under 12:12-h light dark cycle. They were randomly assigned to receive one of the following treatment combinations; 0.9 % saline vehicle (SAL+SAL) as control, SAL + LPS (0.06 μ g kg⁻¹ body weight (BW), *Escherichia coli* 055:B5; Sigma-Aldrich, Co. St

Louis, MO) or insulin (2, 6, 12 or 20 mU kg⁻¹ BW; Human recombinant; Sigma-Aldrich Co. St. Louis MO, USA) + LPS (0.06 μ g kg⁻¹ BW) injected intravenously (i.v.). The treatments were designed such that the first i.v. injection in each treatment combination was given 2 h prior to the second. The sheep were fed 3 % of BW on a concentrate diet made up of 12% crude protein on the morning of an experiment and had free access to water. Treatment was administered to each sheep. The order of treatments was randomized. Jugular blood was sampled at 1 h before and then every 1 h thereafter for 10 h after the insulin bolus. Body temperature was also monitored at the same time as for blood sampling using a rectal digital thermometer.

Blood Metabolite Analysis

Blood samples were placed into vacutainer tubes each containing 100 μ L of 7.5 mg Ethylenediaminetetraacetic acid (EDTA) anticoagulant and centrifuged (1600 x g for 15 min at 4°C) immediately within 10 min of collection. Plasma samples were then stored on ice until transported to the lab. Once in the lab, aliquots of plasma were stored at -20°C until assayed for glucose. Glucose concentrations in the plasma were determined using a commercially available test kit (Autokit Glucose C II kit; Wako Chemicals Inc, Richmond, VA, USA).

Statistical Analysis

The effect of treatment and time on temperature and glucose concentrations were determined using the Generalized Linear Models procedures of SAS (1999) for repeated measures. Mean separation was performed by using the LS Means/PDIFF statements of SAS. Values of $P < 0.05$ were considered significant.

Results

The effect of intravenous administration of saline (SAL+SAL), SAL+LPS or various doses of insulin plus LPS on body temperature in sheep is shown in Table 1. Temperatures for the Saline control sheep

Table 1: Effect of intravenous administration of saline (SAL), lipopolysaccharide (LPS) or insulin and lipopolysaccharide (I+LPS) on body temperature (°C) in sheep (LS Mean ± SEM).

Time (h)	Treatments					
	SAL+SAL	SAL+LPS	2mUI+LPS	6mUI+LPS	12mUI+LPS	38.9±0.1
1	38.9±0.1	38.8±0.1	38.9±0.1	39.0±0.1	38.8±0.1	38.9±0.1
*0	39.1±0.1	39.0±0.1	39.0±0.1	39.1±0.1	39.0±0.1	38.9±0.1
1	38.9±0.2	39.0±0.1	39.1±0.1	39.1±0.1	39.0±0.1	39.0±0.1
#2	39.1±0.1	39.3±0.1	39.2±0.1	39.1±0.2	39.4±0.2	39.2±0.1
3	39.1±0.2 ^a	40.6±0.2 ^b	40.5±0.2 ^b	40.5±0.2 ^b	40.4±0.2 ^b	40.6±0.2 ^b
4	39.1±0.2 ^a	41.2±0.1 ^b	40.9±0.2 ^b	40.8±0.2 ^b	40.7±0.2 ^b	41.0±0.2 ^b
5	39.0±0.3 ^a	41.6±0.3 ^b	40.9±0.3 ^{bc}	41.0±0.3 ^{bc}	40.7±0.3 ^c	41.0±0.3 ^{bc}
6	39.0±0.3 ^a	41.2±0.3 ^b	40.6±0.3 ^{bc}	40.5±0.3 ^{bc}	40.0±0.3 ^c	40.7±0.3 ^{bc}
7	39.1±0.3 ^a	40.5±0.3 ^b	40.2±0.3 ^b	40.2±0.3 ^b	39.6±0.3 ^a	40.2±0.3 ^b
8	39.1±0.2 ^a	40.1±0.2 ^b	39.8±0.2 ^{ab}	39.8±0.2 ^{ab}	39.5±0.2 ^a	39.8±0.2 ^{ab}
9	39.0±0.2 ^a	39.9±0.2 ^b	39.7±0.2 ^b	39.7±0.2 ^b	39.4±0.2 ^{ab}	39.8±0.2 ^b
10	39.0±0.2 ^a	39.6±0.2 ^b	39.5±0.2 ^b	39.7±0.2 ^b	39.3±0.2 ^{ab}	39.5±0.2 ^b

Means within the same row with different superscripts (a,b,c) are significantly different (P<0.05).

-1= initial body temperature 1 h before start of intravenous injections

*0= Time of intravenous administration of insulin

#2= Time of intravenous administration of LPS.

Table 2: Effect of intravenous administration of saline (SAL), lipopolysaccharide (LPS) or insulin and lipopolysaccharide (I+LPS) on plasma glucose concentrations (mg dL⁻¹) in sheep (LS Mean ± SEM).

Time (h)	Treatments					
	SAL+SAL	SAL+LPS	2mUI+LPS	6mUI+LPS	12mUI+LPS	20mUI+LPS
-1	72.5±3.1	71.6±3.1	75.0±3.1	68.2±3.1	73.2±3.1	70.3±3.1
*0	73.0±4.1	70.5±4.1	68.4±4.1	69.0±4.1	64.2±4.1	63.9±4.1
1	71.7±4.5	71.9±4.5	69.2±4.5	63.3±4.5	67.9±4.5	62.1±4.5
#2	70.3±3.5	69.1±3.5	69.2±3.5	63.0±3.5	70.9±3.5	69.0±3.5
3	73.0±5.0	77.2±5.0	70.2±5.0	76.8±5.0	74.6±5.0	72.1±5.0
4	74.2±5.3	77.1±5.3	73.9±5.3	76.9±5.3	79.9±5.3	80.0±5.3
5	76.9±3.9	73.3±3.9	73.7± 3.9	75.0±3.9	78.8±3.9	75.7±3.9
6	73.8±5.1	69.3±5.1	75.7 ±5.1	67.2 ±5.1	69.6±5.1	64.0±5.1
7	73.6±3.5 ^a	58.3 ±3.5 ^b	60.9 ±3.5 ^{ab}	55.4 ±3.5 ^b	55.8±3.5 ^b	55.6±3.5 ^b
8	76.3±4.7 ^a	55.5 ± 4.7 ^{ab}	56.4±4.7 ^{ab}	52.2±4.7 ^b	48.3±4.7 ^b	46.5±4.7 ^b
9	75.5±5.4 ^a	52.8±5.4 ^{ab}	59.5±5.4 ^{ab}	47.9±5.4 ^b	48.0±5.4 ^b	46.6±5.4 ^b
10	80.0 ±6.4 ^a	52.3±6.4 ^b	57.8±6.4 ^{ab}	51.8±6.4 ^b	56.6±6.4 ^{ab}	51.9±6.4 ^b

Means in the same row with different superscripts (a,b,c) are significantly different (P<0.05).

-1= initial body temperature 1 h before start of intravenous injections

*0= Time of intravenous administration of insulin

#2= Time of intravenous administration of LPS.

(SAL+SAL) remained relatively constant throughout the study period ranging from 38.9 ± 0.1 to 39.1 ± 0.1 °C. The administration of LPS to sheep in this study resulted in instant fever. Sheep administered with SAL + LPS had significantly ($P < 0.05$) elevated temperature compared to the saline controls from 1 h to 8 h after LPS administration. Temperatures in the SAL+LPS treated sheep reached a maximum of 41.6 ± 0.1 °C 3 h after LPS administration. This was followed by a gradual decline towards initial normal levels by 8 h post LPS administration. Temperatures in sheep administered with a combination of various doses of insulin (2, 6, 12 and 20 mU) and LPS ($0.06 \mu\text{g kg}^{-1}$ BW) were also elevated from 1 h to 3h after LPS administration. These were followed by steady declines towards normal levels by 8 h after LPS injection. The sheep injected with 12mUI +LPS had significantly ($P < 0.05$) lower body temperature than the SAL +LPS treated sheep from 3 to 6 h post LPS injection. Within the insulin + LPS treatment group the 12mUI +LPS combination was found to significantly reduce ($P < 0.05$) body temperature in sheep to levels similar to the saline controls from 5 to 8 h after LPS administration.

Sheep injected with SAL+LPS or various doses of insulin+LPS had reduced ($P < 0.05$) glucose levels than the saline control sheep from 5 to 8 h post LPS injection.

Discussion

The dose of $0.06 \mu\text{g kg}^{-1}$ BW of LPS used in this study effectively induced typical acute inflammatory symptoms similar to the findings of other studies in sheep (Coleman *et al.*, 1993; Soliman *et al.*, 2001). A fever in response to LPS is presumably due to the prostaglandin-mediated action of cytokines, in particular IL-1 on the hypothalamus (Lohuis *et al.*, 1988; Kluger, 1991). A large body of evidence shows prostaglandin E2 is a principal downstream mediator of fevers produced by LPS and most pyrogenic cytokines, including IL-1 β and IL-6 (Tavares *et al.*, 2006).

The ability of 12mUI+LPS combination within the insulin + LPS treatment group to

significantly reduce body temperature in sheep to levels similar to the saline controls from 5 to 8 h after LPS administration (Table 1) suggests the effectiveness of this treatment combination in alleviating febrile condition in sheep.

The significantly reduced glucose levels in sheep injected with SAL+LPS or various doses of insulin+LPS compared to the saline control sheep from 5 to 8 h post LPS injection (Table 2) could be attributed to the metabolic responses to endotoxin. This includes disruptions in glucose homeostasis (Holzman *et al.*, 1974, Hinshaw, 1976). A transient early hyperglycemia is generally followed by a progression to profound hypoglycemia and a depletion of body carbohydrate reserves (Hinshaw, 1976; Knowles *et al.*, 1986). The hypoglycaemia is due primarily to hypersecretion of insulin by the endotoxic pancreas (Yelich, 2001; Lang, 2001) consequently increasing glucose utilization.

The present study demonstrates the ability of insulin at a dose of 12 mU kg⁻¹ BW to reduce fever in sheep challenged with LPS. Understanding the role of insulin in the inflammatory response to disease in ruminants could help in the development of feeding strategies and novel treatment interventions to increase animal productivity.

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