The effect of glucocorticoids on circulating plasma concentrations of ghrelin in sheep

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ABSTRACT

Activation of the hypothalamic-pituitary-adrenal (HPA) axis to release adrenocorticotropic hormone (ACTH) culminates in glucocorticoid production in sheep. Glucocorticoids alter metabolic function, which suggests concentrations of metabolically important hormones, ghrelin and growth hormone (GH) might be affected by components of the HPA axis. To clarify the relationship between the HPA axis and these hormones in ruminants, 10 Katahdin ewe lambs (1 year old; 43.3 +/- 1.3 kg) received an intravenous injection of ACTH (0.2 µg/kg BW) or saline. In a second experiment, the same lambs received an intravenous injection of the synthetic glucocorticoid, dexamethasone (DEX; 2 mg/kg BW) or saline. Blood samples were collected in 15 min intervals from 1 h before injection to 2 h after. Plasma concentrations of cortisol, ghrelin, and GH were analyzed by radioimmunoassay analysis. Data were tested for effect of treatment (saline or ACTH and saline or DEX), time, and treatment by time interaction using procedures for repeated measures with JMP software (version 10; SAS Inst. Inc.). Cortisol concentrations increased in response to both ACTH and dexamethasone administration ($P < 0.0001$). Neither ghrelin nor GH concentrations were affected by ACTH or DEX administration ($P \geq 0.4372$). These data confirm ACTH influences cortisol secretion, but suggest neither ACTH nor glucocorticoids impact plasma concentrations of ghrelin and GH in sheep.

INTRODUCTION

- The synthetic glucocorticoid dexamethasone is known to stimulate feed intake in sheep (Adams and Sanders, 1992). In fact, dexamethasone is commonly used as a treatment to stimulate intake in ruminants.
- Dexamethasone (and other glucocorticoids) likely stimulate feed intake through a negative feedback loop to inhibit corticotropic releasing hormone (CRH) secretion. CRH is a know inhibitor of feed intake in sheep (Weisinger et al., 2000).
- However, numerous other factors are involved in regulation of feed intake, including ghrelin, which has been demonstrated to stimulate feed intake in sheep (Grouselle et al., 2008). It is possible glucocorticoids could impact factors regulating feed intake other than CRH. Indeed, a recent study in chickens indicated dexamethasone administration can alter expression of neuropeptide Y and agouti-related peptide in the hypothalamus (Liu et al., 2016).
- Therefore, this study was designed to determine if acute changes in glucocorticoids impact circulating concentrations of ghrelin.

MATERIALS AND METHODS

Animals and Maintenance

All animal procedures were approved by the Berry College Institutional Animal Care and Use Committee. Ten open Katahdin ewe lambs were group housed for two weeks prior to the study with water and hay available *ad libitum*. The lambs were group fed approximately 6-kg of pelleted feed (15% crude fiber, 14% crude protein, 2.5% crude fat, 1% (min) – 1.5% (max) calcium, 0.60% (min) – 1.10% (max) NaCl, 0.6 ppm (min) selenium, and 9,000 IU/lb (min) vitamin A) at 16:00. This feeding schedule was maintained to ensure ghrelin concentrations were low prior to blood collection. All procedures occurred in March at a latitude of 34°18′4″N and a longitude of 85°11′20″W with a light-dark period of about 12 h. The lambs were approximately 1 year of age and weighed 43.3 +/- 1.3 kg at experiment 1 and 46.1 kg +/- 1.2 kg at experiment 2.

Experiment 1: Effect of ACTH on plasma ghrelin concentrations

The day before blood collection, 10 ewe lambs were fitted with indwelling jugular cannulas and randomly assigned to one of 2 groups (**CONT1** or **ACTH**). At 16:00 on the day of blood collections, ewe lambs were placed in individual 1.7 m^2 pens, fed, and provided water *ad libitum*. Five mL of blood per ewe lamb per time point were collected starting at 17:00 (time -60) and continued every 15 min for 3 h. Immediately following sample collection at 0 min (18:00), the ewe lambs in the ACTH group (n = 5) were given 0.2 µg/kg BW of ACTH iv while the CONT1 lambs (n = 5) received an equivalent volume of saline iv.

Experiment 2: Effect of dexamethasone on plasma ghrelin concentrations

Experiment 2 was conducted one week after experiment 1. The same 10 ewe lambs were fitted with indwelling jugular cannulas the day prior to blood collection and randomly assigned to one of 2 groups (**CONT2** or **DEX**). At 16:00 on the day of blood collections, ewe lambs were placed in individual 1.7 m^2 pens, fed, and provided water *ad libitum*. Five mL of blood per ewe lamb per time point were collected starting at 17:00 (time -60) and continued every 15 min for 3 h. Immediately following sample collection at 0 min (18:00), the ewe lambs in the DEX group (n = 5) were administered dexamethasone injections (2 mg/kg BW) iv while CONT2 lambs (n = 5) received an equivalent volume of saline iv.

MATERIALS AND METHODS, cont.

Preparation of Plasma Samples and Hormone Analysis

Immediately following collection, blood samples were transferred into collection tubes lined with K2-EDTA anti-coagulant. The samples were immediately initially processed by harvesting plasma following centrifugation at 3,000 x g for 10 min. For ghrelin measurement, 50 µl of 1 *N* HCl and 10 µl of phenylmethylsulfonyl fluoride (10 mg/ml in isopropanol; **PSMF**) were added to 1 mL aliquot of plasma as per the ghrelin assay manufacturer recommendations (EMD Millipore, Billerica, MA) and mixed with a vortex mixer. An additional 1 mL aliquot of plasma was collected for measurement of cortisol and growth hormone. All samples were stored at -20°C and shipped to the University of Missouri for radioimmunoassay analysis.

Plasma concentrations of cortisol were determined in triplicate in a single assay using a commercially available kit (Cortisol Coated Tube RIA Kit, MpBio) with an intra--assay CV of 2.13%. Plasma concentrations of ghrelin were determined in duplicate in a single assay using a commercially available ghrelin RIA kit (Active Ghrelin Kit GHRA-88HK, EMD Millipore). The intra-assay CV was 4.88%. Plasma concentrations of oGH were determined in triplicate in a single assay using procedures previously describe by Powell and Keisler (1995) using NIDDK-anti-oGH-2 AFPC0123080 as the primary antibody. The intra-assay CV was 1.85%.

Statistical Analyses

Three ewe lambs in experiment 1 experienced a loss of jugular cannula integrity before the first collection at time -60 and were removed from the study. The remaining 8 lambs were evenly distributed between ACTH and Saline groups. In experiment 2, to determine the effect of group (CONT or ACTH), time (-60, -45, -30, -15, 0, 15, 30, 45, 60, 75, 90, 105, 120) and group by time interaction on plasma hormone concentration, data were analyzed using procedures for repeated measures with JMP software (version 10; SAS Inst. Inc.). Student's *t* test determined the means separation were the main effect or interaction was significant ($P \leq 0.05$).

RESULTS

Administration of ACTH increased circulating concentrations of cortisol (Figure 1), but did not effect circulating concentrations of ghrelin ($P = 0.7397$; Figure 2). However, circulating concentrations of gherlin did increase with time ($P < 0.0001$) such that circulating concentrations of gherlin were greater at 45, 60, 75, 105, and 120 minutes after time 0 than than time 0 and 60, 45, 30, and 15 minutes before time 0. Administration of ACTH also had no effect on circulating concentrations of GH ($P = 0.6214$; Figure 3).

Administration of dexamethasone resulted in a spike in circulating concentrations of cortisol ($P = 0.<0001$; Figure 4) but did not effect circulating concentrations of ghrelin ($P = 0.4372$); Figure 5). However, circulating concentration of ghrelin was effected by time ($P = 0.0203$). Although there was a tendency for DEX to have greater circulating concentrations of GH ($P = 0.0789$; Figure 6) there was no effect of time ($P = 0.1052$) or treatment by time interaction ($P = 0.4832$) on circulating concentrations of GH.

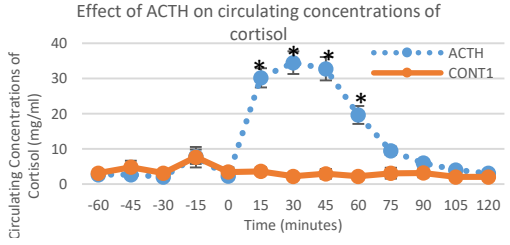

Figure 1. Circulating concentrations of cortisol in ewe lambs that received ACTH I.V. (0.2 µg/kg BW; ACTH; n = 4) or saline solution iv (CONT1; n = 4) immediately following sample collection at time = 0. *ACTH mean plasma cortisol concentrations differed from CONT1 ($P < 0.05$) at same time point.

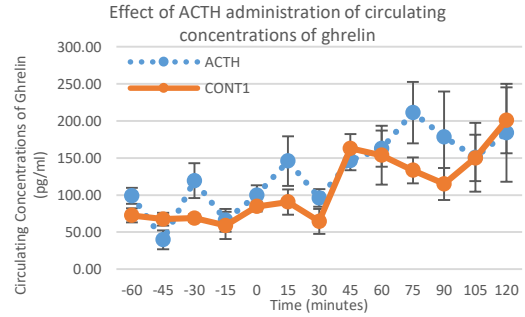

Figure 2. Circulating concentrations of gherlin in ewe lambs that received ACTH I.V. (0.2 µg/kg BW; ACTH; n = 4) or saline solution iv (CONT1; n = 4) immediately following sample collection at time = 0. There was an effect of time ($P < 0.0001$) such that circulating concentrations of gherlin were greater at 45, 60, 75, 105, and 120 minutes than -60, -45, -30, -15, and 0 minutes.

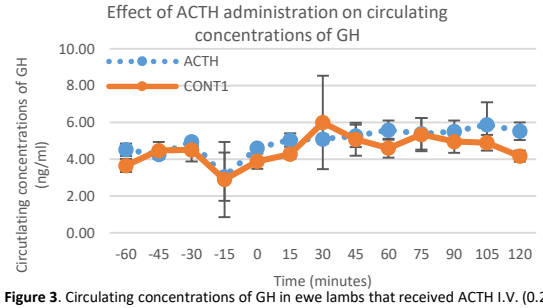

Figure 3. Circulating concentrations of GH in ewe lambs that received ACTH I.V. (0.2 µg/kg BW; ACTH; n = 4) or saline solution iv (CONT1; n = 4) immediately following sample collection at time = 0. There was no effect of treatment ($P = 0.6214$) or treatment by time interaction ($P = 0.9373$). However, there was an effect of time ($P = 0.0093$) such that circulating concentrations of GH were greater at time 30 than time -60, -15, and 0. Furthermore circulating concentrations of GH were also lower at time -60 than time 105 and 75 and at time -15 than every other time except -60.

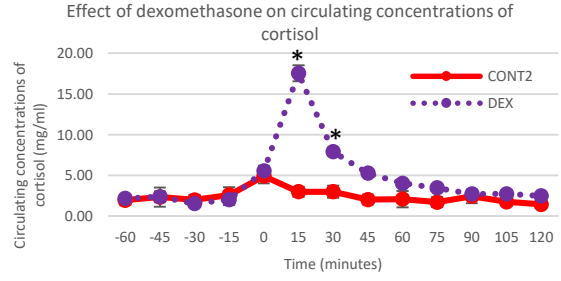

Figure 4. Circulating concentrations of gherlin in ewe lambs that received dexamethasone iv (2 mg/kg BW; ACTH; n = 4) or saline solution iv (CONT2; n = 4) immediately following sample collection at time = 0. *DEX mean plasma cortisol concentrations differed from CONT2 ($P < 0.05$) at same time point.

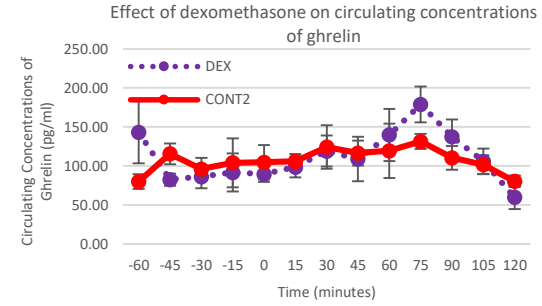

Figure 5. Circulating concentrations of gherlin in ewe lambs that received dexamethasone iv (2 mg/kg BW; ACTH; n = 4) or saline solution iv (CONT2; n = 4) immediately following sample collection at time = 0. There was an effect of time ($P = 0.0203$) such that circulating concentrations of gherlin were greater at 75 minutes than at-60, -45, -30, -15, 0, 15, 45, 105, and 120 minutes and were lower at 120 minutes than -60, 30, 45, 60, 75, and 90 minutes. There was no effect of treatment ($P = 0.7118$) or treatment by time interaction ($P = 0.4372$).

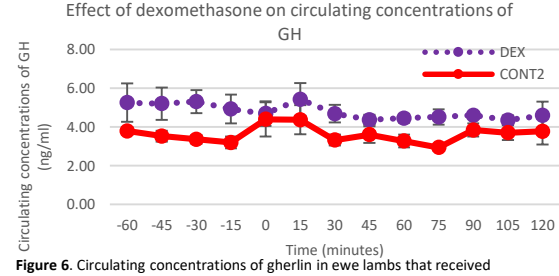

Figure 6. Circulating concentrations of gherlin in ewe lambs that received dexamethasone iv (2 mg/kg BW; ACTH; n = 4) or saline solution iv (CONT2; n = 4) immediately following sample collection at time = 0. There was a tendency for treatment effect ($P = 0.0789$) but there was no effect of time ($P = 0.1052$) or treatment by time interaction ($P = 0.4832$).

Conclusions

Neither ACTH, endogenous cortisol, nor dexamethasone acutely influence circulating concentrations of ghrelin.

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