

Temporal relationships between plasma cortisol, corticosteroid-binding globulin (CBG), and the free cortisol index (FCI) in pigs in response to adrenal stimulation or suppression

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Summary

The objective of this study was to document changes in plasma concentrations of total cortisol, porcine corticosteroid-binding globulin (pCBG), and the free cortisol index (FCI) in pigs over a 6-h period in response to adrenal stimulation or suppression. Twenty-four 8-wk old pigs allotted in equal numbers were administered ACTH, dexamethasone, or saline, and blood samples were collected every 15 min via an indwelling jugular catheter for 1 h prior to and 5 h following treatment administration. Total plasma cortisol increased in ACTH-treated pigs and decreased in dexamethasone-treated pigs within 0.25 and 0.5 h, respectively. In contrast, pCBG concentration was altered in an inverse fashion subsequent to the changes exhibited in total cortisol. FCI reflected the changes observed in total cortisol. These results further document the negative relationship that exists in circulating concentrations of plasma cortisol and pCBG, and illustrate that this association exists under conditions of acute stress in the pig.

Key words: CBG, cortisol, FCI, pig

Introduction

The active form of cortisol in circulation is that which is unbound and that which is loosely bound to albumin, thus biologically available to the cell (Siiteri et al., 1982). The majority of circulating cortisol in humans (Siiteri et al., 1982) and swine (Kattesh et al., 1990) is bound to its specific carrier glycoprotein, corticosteroid-binding globulin (CBG), which both transports and modulates cortisol availability in the circulation. We have reported finding significant changes in circulating CBG concentrations, examined over days or weeks, in pregnant sows (Kattesh et al., 1980), pseudopregnant gilts following cortisol administration (Behrens et al., 1993), and pigs following exposure to heat and social stress (Heo et al., 2005). We recently reported that CBG in young pigs follows a diurnal pattern over a 24 h period (Adcock et al., 2006). Acute changes in circulating CBG in relation to cortisol change has not been examined in swine.

Interpretation of plasma total cortisol concentrations, in situations where CBG changes significantly, does not address the free cortisol fraction and thus may not provide a true representation of its biological impact (le Roux et al., 2003). The free cortisol index (FCI), a ratio of the concentration of plasma cortisol to CBG, has been shown to correlate well with plasma free cortisol in both humans (le Roux et al., 2003) and swine (Adcock et al., 2006).

The objective of this study was to document the changes in plasma total cortisol, CBG and FCI in pigs sampled frequently over a 6-h period in response to adrenal stimulation or suppression.

Methods

Twenty-four 8-wk old female pigs (Premier x QMax 100) selected from 8 litters were assigned to two replicate groups (4 litters/group). Replicate groups of 12 pigs, separated by one week of age, were used due to the frequency of blood sampling and space limitations. One pig from each litter was randomly selected to receive one of the three treatments. Pigs were weaned at 25 d of age and housed in elevated pens (2.4 m x 2.4 m) with slotted floors. Animals were given free access to a commercial diet and water. Artificial lights were provided 13 h, starting at 0630 h, and a red light source was activated during the dark period to aid in blood sampling.

Three days prior to initiation of the experiment, pigs from group one (15.7 ± 2.4 kg) and group two (22.2 ± 2.8 kg) were placed in individual 0.61 m x 1.22 m pens. On the morning of the experiment, pigs were anesthetized and non-surgically fitted with an indwelling catheter. The pigs were returned to their individual pens and assigned to one of three treatments: ACTH ($11.1 \mu\text{g/kg BW}$), dexamethasone ($16 \mu\text{g/kg BW}$) or saline (2.5 ml 0.9% NaCl). All treatments were administered via the indwelling catheter.

Blood sampling began at 1430 h, a minimum of 4 h after the catheter was inserted. Blood samples (5 ml) were collected over a 6-h period every 15 min, beginning at -1 h. Samples were immediately centrifuged at $1520 \times g$ for 15 min, the plasma was removed and stored at -20°C .

Plasma total cortisol concentration was determined by radioimmunoassay, and the concentration of porcine corticosteroid-binding globulin (pCBG) was measured by a direct ELISA. The free cortisol index was calculated using the ratio of plasma total cortisol to pCBG concentration.

Data were analyzed using the MIXED procedure of SAS (SAS Inc., v9.0) for a randomized block design. Samples taken prior to treatment administration were averaged to provide a single baseline value for each pig. Data were represented as least squares means with standard errors, and significant differences were separated using Fisher's Least Significant Difference test.

Results and Discussion

Preliminary analysis indicated there was no effect due to replicate for any of the variables measured; hence, data from both replicates were combined and the results illustrated in Figure 1.

Mean plasma total cortisol concentrations were different ($P < .0001$) due to treatment, time and the interaction of treatment x time. Plasma total cortisol in saline treated pigs decreased ($P < .001$) from baseline values beginning at 0.5 h and remained low for the remainder of the experiment. Pigs administered ACTH exhibited a greater ($P < .001$) cortisol concentration compared to baseline by 0.25 h, which continued until 2.75 h. At 3 h following ACTH injection, plasma total cortisol values returned to baseline and were lower ($P < .001$) than baseline by 3.75 h where they persisted for the remainder of the experiment. In the dexamethasone treated group, plasma cortisol values were lower ($P < .001$) than baseline at 0.5 h and remained suppressed for the duration of the experiment.

Mean plasma pCBG concentrations were similar ($P > .10$) among treatment groups prior to treatment administration. Overall, neither treatment ($P = .67$) nor time ($P = .10$) differences were observed for pCBG; however, there was a strong interaction ($P < .0001$) indicating different treatment responses across time. This interaction was caused by the

overall pattern across time of increasing pCBG for dexamethasone, no changes for saline treated pigs, and a decrease, then increase for ACTH pigs. The pCBG concentrations for saline treated pigs remained similar to baseline values throughout the experiment. For ACTH pigs, pCBG concentrations were lower ($P < .001$) than the mean baseline value from 1.75 to 3.25 h post-treatment, and were similar to the baseline value for the remainder of the experiment. The concentration of pCBG in dexamethasone treated pigs increased ($P < .05$) within 1 h post-injection compared to baseline values and returned to baseline by 4.75 h. At the end of the experiment (5 h), pCBG for ACTH treated pigs was greater ($P < .05$) than that of the dexamethasone or saline treatment groups.

The significance of the observed plasma cortisol-CBG relationship reported here and in previous studies may reside in the calculation of the FCI. The FCI increased following the administration of ACTH resulting from an increase in total cortisol and a decrease in pCBG concentrations. In the dexamethasone treated group, the FCI decreased as a result of a suppression of total cortisol and an increase in pCBG. Arguably, the changes in pCBG over time especially for the ACTH and dexamethasone treated pigs appear trivial, and hence measuring total cortisol alone seems to almost completely predict FCI. However, since approximately 60% of total cortisol in circulation is bound to pCBG in the pig (Kattesh et al. 1990), a change in pCBG could have a significant effect on the amount of biologically available cortisol.

Conclusion

These results confirm our earlier observation of a delayed effect of elevated cortisol on circulating pCBG concentrations in young pigs following ACTH administration. The

FCI may provide a better illustration of temporal changes in the adrenal response in animals than by measuring total plasma cortisol alone.

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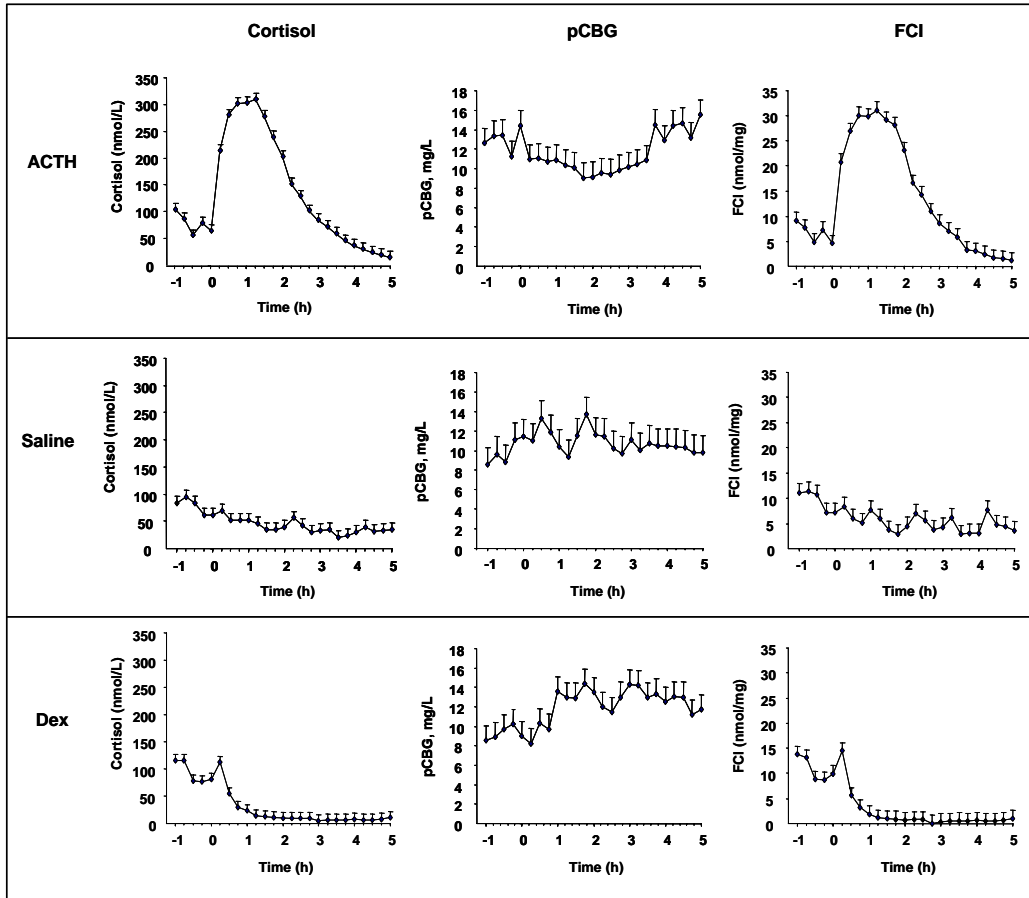


Figure 1. Changes in cortisol, pCBG and FCI in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (Saline; 0.9% NaCl), or dexamethasone (Dex; 16 µg/kg BW). Treatment was administered immediately following 0 h sample. Each point represents the mean value (\pm SE, n = 7 – 8).