A study of pituitary and adrenal function in a disease-stressed ram

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It is well-known that stress inhibits reproduction in domestic as well as laboratory animals. Acute stress in ruminants is associated with increased plasma cortisol concentrations (1,2) and reduced plasma luteinizing hormone (LH) concentrations (3,4). Increases in plasma concentrations of the opioid β-endorphin, which is derived from the same precursor molecule as adrenocorticotropic hormone (ACTH), have also been described in cows and ewes (5,6). The effects of chronic stress are less clear. Plasma concentrations of cortisol were higher in sheep subject to chronic noise stress than in nonstressed controls (1), whereas severe lameness decreased plasma cortisol concentrations (7). Prolonged and repeated electric footshocks decreased mean LH concentrations in ewes, probably by suppressing gonadotrophin-releasing hormone (GnRH) secretion (8,9).

In the course of experiments designed to elucidate the relationship between testicular hormones and pituitary function, the opportunity arose to study the effects of illness on the pituitary-gonadal and pituitary-adrenal axes in a ram.

The ram in question was one of five rams allocated to an experimental group. The animals were 1.5–2.5 years of age, weighed 82 ± 10 kg, and were judged to be in good health at the time of allocation. These studies followed the guidelines of the Canadian Council on Animal Care and were approved by the Ethics Committee of the Faculty of Veterinary Medicine. Under general anesthesia, each animal was castrated and given a 55 × 75 mm subdermal implant made of medical-grade silicone rubber (Silastic 500-1, Dow Corning Corporation, Midland, Michigan, USA) containing crystalline testosterone (Sigma Chemical Company, St. Louis, Missouri, USA). These implants were removed seven days later, under local anesthesia. Blood samples were taken by jugular venipuncture daily at 0800 h for four days prior to castration, and then for five days following removal of the testosterone implants (days 12–16 of the experiment). On the sixth day after implant removal, blood samples were taken through indwelling jugular cannulae (placed at least one day before sampling) every 10 min for 12 h to assess pulsatile LH secretion. The rams were housed together until after the last sampling period. One week after castration, one ram started to show signs of respiratory complaint, while the other four remained in good health. During the week following implant removal, this animal suffered bouts of coughing and had a reduced appetite. Treatment with 1 mg/kg bodyweight ceftiofur (Excenel, Upjohn, Kalamazoo, Michigan, USA) was started on the last day of sampling. After five days of antibiotic treatment, the condition had not improved, and the consulting clinicians recommended that this ram be removed from the group. The animal was humanely killed, and an on-farm postmortem examination revealed extensive caseous lymphadenitis in the thorax but not within the abdomen. No signs of intrascrotal infection had been noted at castration.

All daily samples were assayed for LH and follicle-stimulating hormone (FSH) concentrations using reagents provided by the National Hormone and Pituitary Program (Baltimore, Maryland, USA), the details of which have been reported elsewhere (10); the intra- and interassay coefficients of variation were approximately 5% and 8%, respectively, for both assays, and the sensitivity was 0.2 ng/mL for both assays. Testosterone was measured with an in-house assay employing an antibody that cross-reacted strongly with dihydrotestosterone (>75%) but not with other natural gonadal or adrenal steroids tested (<2%). Samples were extracted with diethyl ether before assay, and values were corrected for procedural losses (mean recovery of added tracer was 87%). The minimum detectable concentration was 0.1 ng/mL, and the intra- and interassay coefficients of variation were 5 and 15%, respectively. Three daily samples before castration and the last three daily samples taken after implant removal (days 14–16) were assayed for ACTH and cortisol (in plastic and glass, respectively) using commercially available double-antibody assay kits employing iodinated tracers (Diagnostic Products Corporation, Los Angeles, California, USA). The sensitivity of these assays was 15 pg/mL for ACTH and 2 ng/mL for cortisol. All samples were run in one assay for each hormone, with an intraassay coefficient of variation of <10%. The data for the healthy animals are presented as means ± SEM, whereas single values are reported for the ill ram. Pulsatile LH secretion was analyzed by an algorithm pulse detection program (11).

Although no bacteriological studies were performed, the animal appeared to have a chronic Corynebacterium infection, of a usually subclinical and nonprogressive nature. Thus the profound suppression of gonadotrophin concentrations observed in this animal (Figure 1) was surprising. After removal of the testosterone implants, there was a rapid increase in plasma LH and FSH concentrations in the healthy rams, forming a typical castration response (10); this response was entirely absent in the ill ram. Although data from one ram are not sufficient to allow a statistical analysis of hormone concentrations, it seems possible from Figure 1 that there was also a suppres-

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Table 1. Mean (± SEM) plasma concentrations of testosterone (T), cortisol, and adrenocorticotropic hormone (ACTH) in a diseased and four healthy control rams before and after castration

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>T (ng/mL)</th>
<th>Cortisol (ng/mL)</th>
<th>ACTH (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before castration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1.6 ± 0.5</td>
<td>8 ± 2</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>Diseased</td>
<td>0.7</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>After castration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>0.1*</td>
<td>3 ± 1</td>
<td>37 ± 6</td>
</tr>
<tr>
<td>Diseased</td>
<td>0.1*</td>
<td>29</td>
<td>41</td>
</tr>
</tbody>
</table>

*Minimum detectable value

Figure 1. Daily plasma LH and FSH concentrations in a diseased ram (●) and mean (± SEM) concentrations in healthy controls (○) for four days prior to castration and placement of a testosterone implant, and then for five days following removal of the testosterone implant. Days are numbered consecutively from the first day of sampling.

This study. A direct effect of ACTH is unlikely, as ACTH appeared not to be affected by disease. An effect of pituitary β-endorphin would also be unlikely, as β-endorphin and ACTH are cosecreted in rams (12). Opioids secreted from the central nervous system may have contributed, as these have been shown to suppress LH secretion in cattle, sheep, and pigs (13). The effects of opioids on FSH secretion in these species, however, have not been well defined (14). The effects of cortisol on gonadotrophin secretion are also not clear; cortisol suppressed the preovulatory LH surge without affecting basal LH concentrations in cows (15), whereas infusions of cortisol did not affect LH release following GnRH injection in rams (16).

The intensive sampling data from this study clearly showed that pulsatile LH secretion was abolished in the diseased ram, whereas mean pulse frequency and amplitude were 9.5 ± 1.1 pulses/12 h and 1.7 ± 0.1 ng/mL, respectively, in the controls (Figure 2). As the rams were killed before these data were analyzed, it was not possible to test pituitary sensitivity to exogenous GnRH; however, an effect of stress on GnRH secretion has been reported in ewes (9). The suppression did appear to be limited to gonadotropes, as ACTH is secreted by the anterior pituitary gland and was not different between the diseased and the healthy animals.

Although no recordings were made of testis size or of sperm number or quality, it is known that disease can inhibit the reproductive process. While the disease was subclinical (before surgery in the present report), there was a tendency for reduced LH, FSH, and testosterone secretion. Thus subclinical disease at the beginning of the breeding season (when higher gonadotrophin concentrations are required for testicular regeneration) may compromise spermatogenesis and lead to economic losses for the animal breeder and, particularly, artificial insemination centers where male animals are concerned.

In summary, disease-related stress markedly suppressed LH and FSH secretion in a ram, which was associated with an increase in cortisol but not ACTH secretion. These data raise the possibility that, in at least some cases, disease-related stress, perhaps in contrast to stress induced by external stimuli, has a greater influence on the pituitary-gonadal axis than on the pituitary-adrenal axis.
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References