Effect of adrenal stimulation on bovine plasma steroids

Hilary Dobson and L. N. Kanchev*

Department of Veterinary Clinical Studies, University of Liverpool, Leahurst, Neston, Wirral, Merseyside L64 7TE, U.K.

Brunner, Donaldson & Hansel (1969) showed that ACTH suppressed corpus luteum formation when administered during Days 2 to 8 of the bovine oestrous cycle. We found that adrenal suppression by betamethasone treatment from Day 10 increased the length of the bovine oestrous cycle: plasma concentrations of oestradiol and androstenedione were unaltered but testosterone levels were reduced by 13% (Kanchev, Dobson, Ward & Fitzpatrick, 1976). The present experiment was undertaken to investigate the effect on plasma steroids of adrenal stimulation achieved by a synthetic adrenocorticotrophin (ACTH).

Five Friesian heifers, 36 months old, were given an intramuscular injection of 2 mg Synacthen Depot (Ciba) equivalent to 200 i.u. ACTH. They were bled by jugular venepuncture before and 2, 6 and 24 h after injection. Plasma was stored at −15°C until analysed. Progesterone and testosterone were measured by the methods described in detail by Kanchev et al. (1976). Cortisol was determined in 0-2 ml samples to which 1 ml ethanol was added. After centrifugation the supernatant was decanted and analysed. The antibody AB2 (raised against cortisol-3-CMO-BSA) was donated by Dr P. H. Rowe and had low cross-reactions with other steroids known to occur in bovine plasma (Cook, Rowe & Dean, 1973). The antibody (0-1 ml at 1:1000) was incubated overnight with 0-1 ml [1,2-3H]cortisol (sp. act. 55 Ci/mmol; Radiochemical Centre, Amersham) and the bound and free fractions were separated with 1 ml 2·5% charcoal. The average (± S.E.M.) recovery of [3H]cortisol added to plasma samples as internal standard in 8 assays was 80·6 ± 4·6%. The water blank was <0·5 ng/ml. When known amounts of cortisol were added, the recovery was 102 ± 17%. The intra- and inter-assay coefficients of variation were 13·3% and 9·9% respectively. Chromatography is not obligatory with this method, as it is with those involving competitive protein binding, isotope dilution or spectrophotometry, and because cortisol is the principal secretary product of the bovine adrenal cortex (Venkataseshu & Estergreen, 1970) this radioimmunoassay should give an accurate assessment of adrenal function.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Cortisol</th>
<th>Testosterone</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>742 ± 261</td>
<td>98 ± 11</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>6</td>
<td>871 ± 234</td>
<td>106 ± 11</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>24</td>
<td>238 ± 38</td>
<td>92 ± 8</td>
<td>96 ± 3</td>
</tr>
</tbody>
</table>

The pretreatment values for cortisol ranged from 3·6 to 10 ng/ml, as reported by others (see Swanson, Hafs & Morrow, 1972). Concentrations of testosterone ranged from 140 to 200 pg/ml, higher values than obtained previously (Kanchev et al., 1976). The reason for this is not known. Progesterone concentrations ranged from 1·0 to 4·0 ng/ml. To facilitate comparison, the pretreatment value for each hormone was expressed as 100% and subsequent values related to this. As shown in Table 1, there was a marked increase in cortisol values after ACTH injection but progesterone and testosterone did not change significantly, suggesting that, although testosterone secretion was suppressed by betamethasone (Kanchev et al., 1976), its production is not under the control of ACTH.

* Present address: Institute of Biology and Pathology of Reproduction, Sofia—13, Bulgaria.
It is possible that ovarian enzymes are depressed by betamethasone or that betamethasone inhibits an adrenal synthetic pathway to testosterone which cannot be stimulated by ACTH.

Venkataseshu & Estergreen (1970) gave the same dose of ACTH to cows but in an aqueous preparation; cortisol was more than doubled after 1 h but had decreased by 2 h, confirming that the depot form used in the present study greatly increases adrenal stimulation. Venkataseshu & Estergreen (1970) also showed that corticosterone values did not change significantly after ACTH, suggesting that the 17-hydroxylating system had been preferentially stimulated.

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References


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