Effect of dexamethasone and acepromazine on plasma androstenedione levels before and after ejaculation of dairy bulls*

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Summary. Acepromazine administered i.v. to 3 bulls 15 min before semen collection blocked seminal emission and coitus-induced androstenedione release. Dexamethasone or saline treatment had no noticeable effect.

Introduction

The phenothiazine tranquillizers can block both seminal emission in man (Freyhan, 1961) and coitus-induced testosterone release in male rabbits (Haltmeyer & Eik-Nes, 1969). The endocrine effects of chlorpromazine, which is probably the most widely utilized of the phenothiazine drugs, are extensive and include blocking the release of FSH, LH, TSH and oxytocin, while increasing the secretion of ACTH, prolactin and vasopressin (see review by de Wied, 1967).

Dexamethasone injection in bulls (Thibier & Rolland, 1976, 1977; Chantaraprateep & Thibier, 1978) results in significant reductions in the circulating levels of LH and testosterone within 3–4 h. The treated bulls showed a diminished responsiveness to LH-RH injection (Thibier & Rolland, 1976; Chantaraprateep & Thibier, 1978), but hCG resulted in a normal pattern of testosterone synthesis and release (Thibier & Rolland, 1977). Male rabbits treated with ACTH showed complete cessation of sexual activity within 60 min (Korányi, Endröczi & Tárnok, 1965/1966), but hydrocortisone had no effect. Behavioural studies in rats have demonstrated that ACTH has effects similar to those seen following barbiturate administration (Gray, Mayes & Wilson, 1971).

Since dexamethasone and the phenothiazine tranquillizers are both capable of altering endocrine, as well as neural elements, it is possible that they may block coitus-induced steroid release, as shown by results from males with hypothalamic lesions (Endröczi, 1962; Kamel & Frankel, 1978). This was examined in the present study using the dairy bull as an experimental model.

Materials and Methods

The 3 dairy bulls used (1 Brown Swiss, 1 Ayrshire and 1 Holstein–Friesian) were 1½–2 years of age. All animals were accustomed to semen collection procedures with an artificial vagina. On

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the day preceding the start of the experiment, indwelling jugular cannulae were inserted. After an adjustment period of 2 h which was used to allow the animals to become accustomed to blood collection procedures and movement of the teaser animal into the collection chute area, pre-treatment blood samples were taken at 15-min intervals for 30 min. Immediately after the last control blood sample, saline (9 g NaCl/l), 10 mg acepromazine maleate (Ayerst Laboratories, New York, U.S.A.) or 15 mg dexamethasone (Azium; Scheering, Kenilworth, New Jersey, U.S.A.) were administered intravenously according to a predetermined 3 x 3 latin square design, with 24-h intervals between treatments. Blood samples were taken at 5-min intervals for 15 min. The bulls were then led from the pen, allowed to mount a teaser animal and semen was collected with an artificial vagina. After ejaculation and return of the bull to his pen, blood samples were again taken at 1, 3, 5, 10, 20 and 30 min after ejaculation.

Cannula patency was maintained by flushing before and after each sample with 5 ml sodium citrate (3-5% w/v). The citrate present in the cannula also served to prevent coagulation of the collected blood sample. Blood samples were kept on ice until plasma was harvested by centrifugation. Individual sample aliquots were frozen over solid CO₂.

Radioimmunoassay of androstenedione

Androstenedione was measured in duplicate samples by the radioimmunoassay method described by Fearnley, Hodgkinson, Holmes & Nordin (1978). The antiserum was raised in rabbits against androstenedione-7a–BSA and was purchased from Miles Laboratories (Elkhart, Indiana, U.S.A.). The antiserum showed a 100% cross-reaction with androstenedione, 70% with 5α-androstane-3,17-dione, 0-5% with testosterone, dehydroepiandrosterone and 11-deoxycorticosterone and <0-1% with other steroids tested. The antiserum, diluted 1:50 with 0-05 m-phosphate-buffered saline (PBS) containing gelatin (1% w/v) and sodium azide (1% w/v), was added (0-5 ml) to each assay tube (steroid extracts and standard curve samples) and the tubes were then incubated at 37°C for 30 min. The tracer, [1,2,6,7(n)-3H]androst-4-ene-3, 17-dione (sp. act. 80–110 Ci/mmol; Amersham Corporation, Arlington Heights, Illinois, U.S.A.) in 0-1 ml PBS, was then added to each tube and incubation continued for 90 min at 37°C. The tubes were then cooled at 4°C for 15 min, after which bound and free androstenedione fractions were

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Saline</th>
<th>Acepromazine</th>
<th>Dexamethasone</th>
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<tbody>
<tr>
<td>-30</td>
<td>275 ± 25</td>
<td>190 ± 6</td>
<td>208 ± 16</td>
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<tr>
<td>-15</td>
<td>233 ± 17</td>
<td>203 ± 42</td>
<td>232 ± 31</td>
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<td>0</td>
<td>225 ± 29</td>
<td>183 ± 18</td>
<td>260 ± 26</td>
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<td>5</td>
<td>212 ± 20</td>
<td>217 ± 8</td>
<td>213 ± 19</td>
</tr>
<tr>
<td>10</td>
<td>207 ± 24</td>
<td>247 ± 15</td>
<td>222 ± 13</td>
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<td>15</td>
<td>187 ± 8</td>
<td>273 ± 14</td>
<td>275 ± 15</td>
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<tr>
<td>16</td>
<td>608 ± 123*</td>
<td>267 ± 9</td>
<td>708 ± 98*</td>
</tr>
<tr>
<td>18</td>
<td>675 ± 101*</td>
<td>208 ± 12</td>
<td>700 ± 73*</td>
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<tr>
<td>20</td>
<td>680 ± 106*</td>
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<td>35</td>
<td>633 ± 121*</td>
<td>200 ± 13</td>
<td>650 ± 15*</td>
</tr>
<tr>
<td>45</td>
<td>683 ± 83*</td>
<td>208 ± 22</td>
<td>642 ± 42*</td>
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* Significantly different from value at 15 min; P < 0-05.
separated using a dextran-coated charcoal suspension. The bound counts were finally extracted into 4 ml toluene-based scintillation fluid.

The sensitivity of the assay was 5 pg. The intra- and inter-assay coefficients of variation were 10·6 and 5·2%, respectively. The recovery of labelled androstenedione after ether extraction was 92%.

Statistical analyses were made with split-plot analysis of variance and Student’s t test.

Results

A split-plot analysis of variance of the data (Table 1) revealed a significant ($P < 0·05$) interaction between the drug treatments and the time of blood sampling. Acepromazine had no effect on circulating androstenedione levels, but these bulls, although thrusting in a normal fashion, emitted no semen into the artificial vagina. In bulls treated with saline or dexamethasone, androstenedione was significantly higher ($P < 0·05$) by 1 min after ejaculation and remained high for the next 30 min. There were no noticeable changes in reproductive behaviour or seminal emission after saline or dexamethasone treatment.

Discussion

The release of androstenedione into the peripheral circulation after ejaculation by the saline- and dexamethasone-treated animals is consistent with previous studies of the bull (Katongole, Naftolin & Short, 1971; Smith, Mongkonpunya, Hafs, Convey & Oxender, 1973; Weathersbee & Lodge, 1976). Although we monitored only androstenedione levels, testosterone, progesterone and oestrogen levels probably also increased, based on testicular arterial and venous cannulation studies in the bull (Amann & Ganjam, 1976). The immediate nature of steroid release after sexual stimulation of the bull, in this and other studies (Smith et al., 1973; Weathersbee & Lodge, 1976), would seem to argue against Lindner’s (1961) concept that the bovine testes does not maintain any preformed hormone stores.

The dexamethasone-treated males experienced neither a noticeable change in reproductive behaviour, a finding in agreement with results for male rabbits injected with hydrocortisone (Koranyi et al., 1965, 1966), nor alterations in sperm output. Previous studies with bulls indicate that had the treatment been administered several hours before ejaculation, it might have blocked or severely diminished any coitus-induced steroid secretion (Thibier & Rolland, 1976, 1977; Chantaraprateep & Thibier, 1978), but this does not happen in male rabbits in which testosterone levels are artificially depressed by injection of oestradiol benzoate as significant changes in blood testosterone levels still occur after ejaculation (Hilliard, Pang, Penardi & Sawyer, 1975).

The injection of bulls with acepromazine before semen collection not only blocked seminal emission (Freyhan, 1961) but interfered with the normal release of steroid, as found for male rabbits treated with chlorpromazine (Halmeyer & Eik-Nes, 1969). Although the phenothiazine tranquilizers cause alterations in a number of endocrine responses (de Wied, 1967), we suggest that the blockage of oxytocin secretion is the change which underlies our results. Injection of oxytocin into male rats causes increased rates of testicular perfusion (Berde, 1964), and in bulls immediate changes in circulating testosterone levels can occur (Weathersbee & Lodge, 1975), analogous to those seen following ejaculation.

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References


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