Synergetic effect of pimozide and thyrotropin releasing hormone on prolactin and thyrotropin release during the drying off of ewes

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Abstract

The effect of pimozide and/or TRH was investigated on plasma prolactin, thyrotropin, T4 and T3 and udder distension in 38 ewes during drying off by feed restriction. The effect of daily injections of 2 mg pimozide (s.c.), combined or not with TRH stimulation (200 µg, i.v.) on three different days of the drying off period was examined. Blood samples were taken twice daily in each group for 9 days, while blood sampling on the days of TRH injection was also performed at 0, 15, 30 min, and 1, 2 and 4 h post-injection. Plasma was assayed for PRL, TSH, T4 and T3 levels. Udder distension and mastitis incidence were recorded at the end of the drying off period. TRH and pimozide both resulted in elevated plasma PRL levels and acted in a synergetic way. Udder distension and the incidence of mastitis was only influenced by pimozide. The TSH as well as the T3 response to TRH was increased in ewes under a continuous influence of pimozide and T3 peaks following TRH injection occurred earlier than T4 peaks. The higher effect of pimozide upon TRH stimulated PRL and TSH release at day 8 compared to days 0 and 3 indicates a progressive involvement of dopamine on the inhibition of PRL and the sensitivity of the thyrotrophs to TRH during drying off.

Keywords: Prolactin; Ewe; Drying off; Pimozide; TRH

1. Introduction

The control of the prolactin (PRL) secretion in ewes is mainly dopaminergic, as dopamine is the main hypothalamic PRL-inhibiting factor (Ben-Jonathan, 1985), while Thyrotropin Releasing Hormone (TRH) is suggested to be the most important PRL-stimulatory agent (Thomas et al., 1988). Pimozide, a dopamine antagonist, is able to suppress the action of dopamine and to stimulate the prolactin concentration as demonstrated in rats and humans (Clemens et al., 1974; Ojeda and McCann, 1974). In humans a daily injection of 2 mg pimozide results in a continuously augmented plasma PRL level (Collu et al., 1975). Also thyroid hormones and hence TSH and its controlling factor TRH are able to stimulate lactation (Fulkerson, 1981). Since TRH administration is increasing
PRL concentration for a much more prolonged time in pregnant and lactating ewes than in neonatal or pre-puberal lambs (Peeters et al., 1992), changes in PRL sensitivity for its stimulating hypothalamic factors, may be expected. Moreover, a decrease in PRL concentration accompanies drying off in ewes (Buys, personal communication). Therefore, the effect of pimozide and/or TRH was investigated on plasma PRL, TSH, T3 and T4. Since stimulation of PRL secretion during drying off may slow down the drying off process, influences of treatments on udder distension and the development of mastitis were followed simultaneously.

2. Materials and methods

2.1. Animals

The experiment was performed on ewes (cross-breeds of Suffolk and Flemish Milksheep) with comparable milk productions. All ewes lambed within a 2 weeks period and had one sucking lamb at the start of the experiment. At 5–7 weeks postpartum all lambs were weaned (day before the start of the experiment (D 0). Consequently the ewes were dried off by giving no feed or water for the following 3 days (D 1–3). The following 5 days the ewes only had access to hay and drinking water (D 4–8).

2.2. Experimental set-up

Ewes of different genotypes were equally divided over five experimental groups. Since pimozide (Orap®, Janssen Pharmaceutica) has a long term action and TRH is more short term working, different injections were given with different frequency. As a consequence blood sampling frequency did vary between experimental groups. From day 0 until day 9 at 8.00 h groups B and C received a daily injection of 2 mg pimozide per ml ethanol 80% (s.c.) while group A was vehicle treated with ethanol 80%. Groups C–E were injected on three different days of the drying off period respectively with 200 µg TRH (i.v.) for C and D and saline for E. Blood samples were taken twice daily in each group (at 8.00 and 20.00 h) before giving the injections. At days 0, 3 and 8 of the drying off period, TRH or saline injections were given at 13.00 h and blood samples were taken at 0, 15, 30 min, and 1, 2 and 4 h after injection in groups C–E. Blood was collected in heparinized tubes and immediately centrifuged (10 min, 3000 rpm). The plasma was recovered and stored at −20°C until further analysis.

2.3. Analytical methods

The o-PRL and o-TSH concentrations were determined by a double antibody radio immunoassay (Peeters et al., 1992). The reagents for the o-PRL RIA and for the o-TSH RIA were supplied by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK, Baltimore, USA). The purity of the antigens (NIDDK-o-PRL-I-2 and NIAMMD-o-TSH-I-1) and the specificity of the antisera (NIDDK-anti-oPRL-2 and NIAMMD-anti-o-TSH-1) was ensured by the supplier. The tracer was obtained by radio-iodination of the antigen using the iodogen method (Fraker and Speck, 1978). For the o-TSH RIA the intra- and interassay coefficients were respectively, 3.3 and 8.6%. All samples were determined within one o-PRL assay with an intra-assay coefficient of 3.9%.

The T4 concentration was measured by a RIA procedure using standards supplied by Byck Belga, tracer T4 125I (Amersham International, UK) and an antiserum that was prepared in the own laboratory. The intra- and interassay coefficients were respectively, 4.2 and 3.1%. The cross reactivity with T3 was 3.5% and T4 levels ranging from 4.02 to 129 nmol/l could be measured. Standards and antisera used for the T3 RIA were supplied by Byck Belga and tracer T3 125I by Amersham International. The intra- and interassay coefficients were respectively, 1 and 10% and detection limits were 0.192 and 6.16 nmol/l. Statistical analysis of the data was performed using the ‘Repeated Measurements Analysis of Variance’ and the ‘LS Means test’ of the General Linear Models Procedure (SAS Institute Inc., 1985).

On day −1 a milk sample was taken from each udder gland of all ewes for bacterial analysis. Throughout the experiment the udders of the ewes were daily examined on udder distension and clinical signs of mastitis. Ewes with a very distended udder on day 8 were milked and treated with antibiotics (dry-Cow, Upjohn) to prevent mastitis. Milk samples of these ewes were analysed for the occurrence of pathogenic bacteria that can cause mastitis.
3. Results

3.1. Prolactin

The plasma PRL level decreased during the drying off period (time: $P < 0.001$) and this variation was dependent on the given treatment (interaction time × treatment: $P < 0.05$). The overall plasma PRL concentration was higher in pimozide treated ewes ($P < 0.001$). Daily injections of pimozide (group B) resulted in continuously elevated plasma PRL concentrations as compared to the control ethanol group (group A). Although no difference was found in overall mean plasma PRL level between ewes treated with pimozide + TRH and those treated with pimozide only, the daily variation of the plasma PRL level during the drying off period was clearly different (Table 1). In ewes treated with pimozide + TRH (group C) or with TRH only (group D) the PRL level was higher on the days TRH was administrated, while it was even lower than in the control groups (A and E) on the days in between ($P < 0.05$). TRH increased the PRL response to pimozide on days 3 and 8 (Table 1, $P < 0.05$). A different pattern in variations in PRL levels throughout the drying off period was observed. In both control groups (A and E) the PRL level in plasma was higher on the day before drying off as compared to the following days ($P < 0.01$).

Variations in plasma PRL concentration immediately following injection of TRH (Fig. 1) reveals some strong interactions: time; $P < 0.001$; day × treatment; $P < 0.001$; time × day; $P < 0.001$ and day × treatment $P < 0.001$). On days 3 and 8 the mean PRL concentration was the highest in ewes treated with TRH and pimozide ($± 640 \mu g/l$), lower in those treated only with TRH ($± 494 \mu g/l, P < 0.01$) and still lower in control ewes ($± 179 \mu g/l, P < 0.001$). In control ewes no difference was found between days 0 and 3, nor between days 3 and 8, but the mean PRL value was higher on day 0 compared to day 8 ($P < 0.001$). Ewes treated with TRH or with TRH + pimozide showed a significantly higher PRL level on day 8 and ($P < 0.05$ and $P < 0.001$) as compared to days 0 and 3.

Basal PRL levels, as measured before injection, were significantly lower in ewes only treated with TRH as compared to both other groups of ewes ($P < 0.001$) (Fig. 1). Although on day 0 no difference in PRL increase between ewes treated with TRH and pimozide and those treated with TRH only was found, on days 3 and 8 the response was higher in ewes under a continuous influence of pimozide ($P < 0.001$).

3.2. Thyrotropin

The overall picture of TSH showed a slight variation in levels in all groups indicating a slight but transient increase during the first days of the drying off period followed by a rather decreased level towards the end of the period (Table 2). Pimozide by itself had

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Table 1
Daily mean plasma PRL concentration ($\mu g/l ± S.E.M.$, blood samples at 8.00 and 20.00 h) during drying off in ewes treated with pimozide (2 mg in 1 ml ethanol 80% s.c. per day at 8.00 h) and/or TRH (200 $\mu g$ in 1 ml saline i.v. on days 0, 3 and 8 at 13.00 h) and in vehicle treated control ewes.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control-ethanol (A)</th>
<th>Pimozide (B)</th>
<th>Pimozide + TRH (C)</th>
<th>TRH$^d$ (D)</th>
<th>Control-saline (E)</th>
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<tbody>
<tr>
<td>0</td>
<td>313$^a$ ± 47</td>
<td>231$^a$ ± 24</td>
<td>441$^b$ ± 136</td>
<td>468$^a$ ± 96</td>
<td>460$^b$ ± 96</td>
</tr>
<tr>
<td>1</td>
<td>205$^{a,b}$ ± 46</td>
<td>259$^a$ ± 38</td>
<td>137$^b$ ± 36</td>
<td>89$^b$ ± 11</td>
<td>158$^b$ ± 22</td>
</tr>
<tr>
<td>2</td>
<td>195$^{a,b}$ ± 59</td>
<td>283$^b$ ± 40</td>
<td>97$^b$ ± 16</td>
<td>58$^b$ ± 5</td>
<td>113$^a$ ± 18</td>
</tr>
<tr>
<td>3</td>
<td>155$^{a,b}$ ± 38</td>
<td>233$^a$ ± 64</td>
<td>463$^a$ ± 146</td>
<td>218$^a$ ± 69</td>
<td>158$^b$ ± 98</td>
</tr>
<tr>
<td>4</td>
<td>153$^{a,b}$ ± 33</td>
<td>339$^{a,b}$ ± 175</td>
<td>56$^b$ ± 4</td>
<td>44$^b$ ± 3</td>
<td>90$^b$ ± 12</td>
</tr>
<tr>
<td>5</td>
<td>184$^{a,b}$ ± 30</td>
<td>190$^b$ ± 22</td>
<td>64$^a$ ± 9</td>
<td>53$^b$ ± 5</td>
<td>113$^a$ ± 25</td>
</tr>
<tr>
<td>6</td>
<td>217$^{a,b}$ ± 97</td>
<td>465$^b$ ± 182</td>
<td>129$^b$ ± 63</td>
<td>78$^b$ ± 17</td>
<td>105$^b$ ± 22</td>
</tr>
<tr>
<td>7</td>
<td>113$^{a,b}$ ± 17</td>
<td>297$^{a,b}$ ± 170</td>
<td>65$^b$ ± 9</td>
<td>79$^b$ ± 25</td>
<td>109$^b$ ± 20</td>
</tr>
<tr>
<td>8</td>
<td>89$^b$ ± 12</td>
<td>140$^a$ ± 21</td>
<td>496$^b$ ± 155</td>
<td>231$^a$ ± 54</td>
<td>102$^b$ ± 24</td>
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<tr>
<td>9</td>
<td>67$^b$ ± 7</td>
<td>278$^{a,b}$ ± 156</td>
<td>187$^b$ ± 121</td>
<td>144$^{b}$ ± 92</td>
<td>93$^b$ ± 17</td>
</tr>
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</table>

$^{a,b,c}$ Within one group, values with different superscripts are significantly different ($P < 0.05$).
$^d$ TRH stimulated values on days 0, 3 and 8 are in bold.
no marked influence on this time pattern but the peak values following a TRH injection were significantly increased in the pimozide treated group (D) at days 3 and 8 ($P < 0.001$).

The immediate TSH response following a TRH or a TRH + pimozide treatment revealed the same picture. TSH-levels increased to up to 20 μg/l at days 0, 3 and 8 after TRH injection (between 15 min and 1 h after

Fig. 1. Effect of TRH (200 μg/ml) or saline on plasma PRL concentrations (μg/l) at various times after the injection on three different days of the drying off period in control ewes or ewes treated with pimozide (2 mg/day). Within one day and treatment values with the same indices are not significantly different for $P < 0.05$. 
control ones (P<0.01) dependent on the treatment (P<0.001). Also the mean plasma T4 level differed among the five experimental groups (treatment: P<0.001). Ewes that were treated with pimozide only, showed higher plasma T4 levels than control ewes (P<0.005). In both groups of TRH treated ewes plasma T4 concentrations were however lower than in control ewes (Table 3). Also only in these two groups the plasma T3 level varied significantly during the experimental period showing a decrease from day 1 on. On the days of the TRH treatment there was a slight increase in plasma T4 although these peak values remained still lower (day 3) or as high (day 8) as the level in control ewes at the same moment. In each group the plasma T3 level decreased during drying off, but this decrease was more pronounced in both groups of ewes treated with TRH than in the three other groups (Table 4). The mean plasma T3 level was lower in both TRH treated groups (P<0.001) but on the days of TRH injection

### 3.3. Thyroid hormones

The plasma T4 and T3 concentrations varied during the drying off period (time: P<0.001) dependent on the treatment (P<0.001). Also the mean plasma T4 level differed among the five experimental groups (treatment: P<0.001). Ewes that were treated with pimozide only, showed higher plasma T4 levels than control ones (P<0.005). In both groups of TRH treated ewes plasma T4 concentrations were however lower than in control ewes (Table 3). Also only in these two groups the plasma T3 level varied significantly during the experimental period showing a decrease from day 1 on. On the days of the TRH treatment there was a slight increase in plasma T4 although these peak values remained still lower (day 3) or as high (day 8) as the level in control ewes at the same moment. In each group the plasma T3 level decreased during drying off, but this decrease was more pronounced in both groups of ewes treated with TRH than in the three other groups (Table 4). The mean plasma T3 level was lower in both TRH treated groups (P<0.001) but on the days of TRH injection

### Table 2

Daily mean plasma TSH concentration (μg/l ± S.E.M., blood samples at 8.00 and 20.00 h) during drying off in ewes treated with pimozide (2 mg in 1 ml ethanol 80% s.c. per day at 8.00 h) and/or TRH (200 μg in 1 ml saline i.v. on days 0, 3 and 8 at 13.00 h) and in vehicle treated control ewes

<table>
<thead>
<tr>
<th>Day</th>
<th>Control-ethanol (A)</th>
<th>Pimozide (B)</th>
<th>Pimozide + TRH (C)</th>
<th>TRH (D)</th>
<th>Control-saline (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.92a,b ± 0.72</td>
<td>6.57a,b,c ± 0.66</td>
<td><strong>11.17</strong> ± 2.49</td>
<td><strong>11.84</strong> ± 1.80</td>
<td>6.41a ± 0.80</td>
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<td>1</td>
<td>8.07a,b ± 1.33</td>
<td>8.15a,b ± 0.82</td>
<td>8.48b ± 1.70</td>
<td>6.60b,c ± 0.52</td>
<td>6.61a ± 0.61</td>
</tr>
<tr>
<td>2</td>
<td>8.45a ± 1.06</td>
<td>9.60a ± 1.10</td>
<td>9.91b ± 2.50</td>
<td>8.66a,b ± 1.12</td>
<td>6.63a ± 0.51</td>
</tr>
<tr>
<td>3</td>
<td>6.76a,b ± 0.75</td>
<td>7.93a,b ± 1.16</td>
<td><strong>15.04</strong> ± 3.67</td>
<td><strong>10.88</strong> ± 1.09</td>
<td>6.25a ± 0.87</td>
</tr>
<tr>
<td>4</td>
<td>7.52a,b ± 0.84</td>
<td>6.71b,c ± 0.68</td>
<td>7.34b,c ± 1.52</td>
<td>6.68b,c ± 0.74</td>
<td>6.11a ± 0.67</td>
</tr>
<tr>
<td>5</td>
<td>6.62a,b ± 0.77</td>
<td>6.20b,c ± 0.94</td>
<td>6.87b,c ± 0.97</td>
<td>5.82b,c ± 0.33</td>
<td>5.75a ± 0.63</td>
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<tr>
<td>6</td>
<td>5.75a,b ± 0.41</td>
<td>4.80b ± 0.41</td>
<td>5.47b ± 0.62</td>
<td>5.78b,c ± 0.49</td>
<td>5.06a ± 0.60</td>
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<tr>
<td>7</td>
<td>5.89a,b ± 0.41</td>
<td>4.41a ± 0.31</td>
<td>5.08b ± 0.45</td>
<td>5.96b,c ± 0.46</td>
<td>5.30a ± 0.36</td>
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<tr>
<td>8</td>
<td>5.47b ± 0.37</td>
<td>4.74b ± 0.40</td>
<td><strong>12.09</strong> ± 2.73</td>
<td><strong>7.86</strong> ± 1.07</td>
<td>5.79a ± 0.93</td>
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<tr>
<td>9</td>
<td>5.90a,b ± 0.50</td>
<td>4.93b ± 0.35</td>
<td>6.59b ± 1.43</td>
<td>4.69b ± 0.30</td>
<td>5.15a±1.43</td>
</tr>
</tbody>
</table>

**Within one group, values with different superscripts are significantly different (P < 0.05).**

### Table 3

Daily mean plasma T4 concentration (nmol/l ± S.E.M., blood samples at 8.00 and 20.00 h) during drying off in ewes treated with pimozide (2 mg in 1 ml ethanol 80% s.c. per day at 8.00 h) and/or TRH (200 μg in 1 ml saline i.v. on days 0, 3 and 8 at 13.00 h) and in vehicle treated control ewes

<table>
<thead>
<tr>
<th>Day</th>
<th>Control-ethanol (A)</th>
<th>Pimozide (B)</th>
<th>Pimozide + TRH (C)</th>
<th>TRH (D)</th>
<th>Control-saline (E)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>66.8a ± 2.4</td>
<td>58.9a ± 3.5</td>
<td><strong>61.9</strong> ± 5.8</td>
<td><strong>66.6</strong> ± 6.3</td>
<td>64.0a ± 4.9</td>
</tr>
<tr>
<td>1</td>
<td>59.5a,b ± 2.1</td>
<td>65.7a ± 4.2</td>
<td>51.5b,c ± 4.1</td>
<td>64.9ab ± 3.5</td>
<td>65.9a ± 4.1</td>
</tr>
<tr>
<td>2</td>
<td>60.4a,b ± 2.7</td>
<td>72.5a ± 4.8</td>
<td>36.4a ± 4.0</td>
<td>44.5cd ± 3.0</td>
<td>64.9a ± 3.5</td>
</tr>
<tr>
<td>3</td>
<td>65.4a,b ± 2.8</td>
<td>80.4a ± 4.1</td>
<td><strong>48.4</strong> ± 6.1</td>
<td><strong>51.3</strong> ± 5.5</td>
<td>69.3a ± 2.6</td>
</tr>
<tr>
<td>4</td>
<td>64.9ab ± 2.2</td>
<td>75.9a ± 5.2</td>
<td>41.8ab,c ± 4.1</td>
<td>45.9ab,c ± 4.4</td>
<td>63.7a ± 2.1</td>
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<td>63.2a,b ± 2.3</td>
<td>76.1a±4.5</td>
<td>35.6a ± 4.1</td>
<td>35.1a ± 3.5</td>
<td>62.4a ± 2.2</td>
</tr>
<tr>
<td>6</td>
<td>59.1ab ± 3.3</td>
<td>71.8a ± 4.0</td>
<td>42.7b,c ± 5.0</td>
<td>34.7a ± 4.5</td>
<td>63.3a ± 2.8</td>
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<tr>
<td>7</td>
<td>59.1ab ± 2.3</td>
<td>71.6a ± 3.7</td>
<td>40.6b,c ± 4.8</td>
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<td>64.5a ± 3.3</td>
</tr>
<tr>
<td>8</td>
<td>57.8a,b ± 2.6</td>
<td>75.2a ± 2.6</td>
<td><strong>59.2</strong> ± 7.0</td>
<td><strong>54.4</strong> ± 7.1</td>
<td>69.1a ± 3.7</td>
</tr>
<tr>
<td>9</td>
<td>54.8b ± 2.3</td>
<td>72.8a ± 2.8</td>
<td>51.5ab,b ± 4.5</td>
<td>53.4a ± 3.7</td>
<td>70.3a ± 3.5</td>
</tr>
</tbody>
</table>

**Within one group, values with different superscripts are significantly different (P < 0.05).**

**TRH stimulated values on days 0, 3 and 8 are in bold.**
the T3 level was as high or higher in TRH treated ewes as in control and pimozide treated ones.

TRH administration always resulted in an increase in plasma T4. While the increase in T4 was only significant 4 h after TRH or TRH\(^{‡}\) treatment, the increase in T3 started earlier, mostly 1 h after TRH injection with or without pimozide, and remained high until at least 4 h later. Only on day 8 the peak value in T3 concentration was higher in ewes treated with pimozide and TRH than in those only receiving TRH (\(P < 0.001\)). While on days 0 and 3, T3 increases upon TRH were not different whether the ewes were treated or not with pimozide, and values were similar to those of day 8 treated with TRH only.

3.4. Udder distension

Treatment with pimozide increased the percentage of ewes per group that had to be milked on day 8 because of a high degree of udder distension (87% compared to 43%). TRH did not influence udder distension.

3.5. Mastitis

At the start of the drying off period no ewe showed clinical signs of mastitis in any gland and this was still the case in control ewes (groups A and E) and in those treated with TRH only (group D) at the end. No difference was found between groups A, D and E concerning the number of glands in which pathogenic bacteria were detected. The only pathogens detected were *Staphylococcus aureus* and *Staphylococcus xylosus* which were present in 11% of the milk samples before and in 15% after drying off in these three groups. In ewes that had been treated with pimozide the number of glands in which these intramammary infections were found increased from 36% at the beginning to 64% at the end of the drying off period. In both groups of ewes treated with pimozide however (groups B and C), 47% of the glands were inflamed after drying off.

4. Discussion

Our results clearly demonstrate a differential effect of TRH, the hypothalamic PRL stimulating factor and of pimozide, a dopamine antagonist, on the plasma PRL level during drying off. Administration of pimozide results in a continuously elevated PRL level that can be explained by the long term action of pimozide as a dopamine receptor blocker (Collu et al., 1975). The coincidence between elevated PRL levels at the end of lactation and a higher milk production has been observed earlier in goats (Vandeputte-Van Mersom and Peeters, 1982). In these experiments the elevated plasma PRL level was reached by implants of perphenazine in the median eminence of goats. As pimozide, perphenazine is a dopamine antagonist, that reduces the decrease in milk production at the end of lactation. Also i.v. injections of perphenazine...
stimulate the PRL secretion (McNeilly and Lamming, 1971). Although Morag et al. (1971) found no galactopoietic effect of i.m. injections of perphenazine, this could be due to the extremely high doses used in their experiments (Vandeputte-Van Messom and Peeters, 1982). In our experiment we found an obvious effect of administration of a dopamine antagonist, pimozide, on milk production in ewes during drying off as estimated by udder distension. The stimulated milk production during drying off is reflected by a higher incidence of clinical mastitis, as was observed earlier (Watson and Buswell, 1984). It was found that while half of the glands of pimozide treated ewes were inflamed after drying off, no other ewe suffered from this problem. All intramammary infections were caused by Staphylococci and the number of glands in which a high concentration of these bacteria was present almost doubled during drying off in pimozide treated ewes while it remained almost unchanged in control ewes and those treated with TRH only. These results clearly indicate that the decrease in PRL concentration occurring at drying off is necessary for a decrease in milk production that is at its turn important to avoid mastitis after drying off. Therefore, a dopamine agonist could be beneficial for a more rapid decrease in PRL and hence in milk production during drying off. This can even limit the feed restriction period for these ewes which may be more compatible with animal welfare regulations.

Injection of TRH on days 0, 3 and 8 of the drying off period results on each day in an increase in plasma PRL and TSH levels. TRH is a potent stimulator of both hypophysial hormone secretions in sheep (Debeljuk et al., 1973; Fell et al., 1974; Wright et al., 1981). The basal as well as the TRH stimulated PRL levels are however higher in ewes that are also treated with pimozide because of the lack of action of dopamine as PRL inhibiting factor (Peters et al., 1981), since administration of dopamine inhibits the PRL response to TRH in sheep (Elsasser and Bolt, 1987). The higher effect of TRH upon PRL response to pimozide on day 8 of drying off compared to days 0 and 3 indicates a progressive involvement of dopamine in the inhibition of PRL during the drying off period. On the other hand dopaminergic stimuli also decrease the TSH secretion in rats (Krulich et al., 1977) and humans (Scanlon et al., 1979). In humans injection of dopamine inhibits the PRL and TSH responses to TRH (Besser et al., 1972) and blocking the dopamine receptor increases PRL and TSH levels (Healy and Burger, 1977). Therefore, dopamine and TRH have an antagonistic action on PRL and TSH secretion in humans (Burrow et al., 1977). A similar mechanism in sheep could explain the higher PRL response to TRH in pimozide treated animals, since also the TSH peaks reached following TRH are higher in ewes receiving pimozide. Moreover, the increased TSH response to TRH in pimozide treated ewes on days 3 and 8 also indicates a progressive dopaminergic inhibition of the sensitivity of the thyrotrophs to TRH in the ewe. In contrast to our results, however, Elsasser and Bolt (1987) found no effect of dopamine on the basal or TRH stimulated TSH secretion in sheep.

The peak in plasma T₃ concentration occurring after TRH stimulation appears earlier, after 1 h, than the maximal T₄ level, after 4 h. Also Peeters et al. (1992) observed an increase in plasma T₃ prior to the increase in plasma T₄ in prepuberal lambs and adult ewes. The preferential increase in plasma T₃ is probably due to a preferential T₃ secretion in the thyroid gland since it has been proven in several mammals (rats: Erickson et al., 1982; mice: Wu et al., 1985) that TSH stimulates the intrathyroidal T₄ to T₃ conversion. As observed earlier the plasma T₃ level decreases during the drying off period (Buys et al., 1995), and also the T₃ response to TRH is lower at the end of this period as compared to the beginning. The T₃ response to TRH is however increased by pimozide, which can be explained by the higher TSH response to TRH in these ewes.

As a conclusion this study linked the decrease in milk secretion during drying off with the PRL secretion in this period. Dopamine plays an important role in both and pimozide as a dopamine antagonist increases both the TSH and PRL responses upon TRH as well as the incidence of milk secretion during drying off may be strengthened by dopamine agonists, thus preventing udder problems and possibly substituting partly for a severe food restriction of ewes.

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References


