Effect of naloxone on the plasma levels of LH, FSH, prolactin and testosterone in Beetal bucks

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Abstract

Ten adult male Beetal goats were used for the study to elucidate the modulation of gonadotrophin, prolactin and testosterone secretion by endogenous opioid peptides. An indwelling catheter was placed in the jugular vein of each buck 20 h before the onset of the experiment. Bucks were divided randomly into two groups: Group I (n = 5) received naloxone at a dose rate of 1 mg/kg body weight (BW) and Group II (n = 5) received naloxone at a dose rate of 2 mg/kg BW intravenously. Blood samplings were done from 2 h before treatment until 2 h after treatment at 15 min intervals. Blood samples were quantified for plasma LH, FSH and prolactin concentration using a heterologous double antibody radioimmunoassay (RIA) and testosterone concentration was quantified by coat-a-count RIA kit. The mean plasma LH levels during pretreatment phase were 0.41 ± 0.03 ng/ml in Group I and 0.44 ± 0.02 ng/ml in Group II which significantly (p < 0.05) increased to 0.91 ± 0.05 ng/ml in Group I and 1.53 ± 0.07 ng/ml in Group II. The mean plasma FSH levels did not show a difference in pre- and post-treatment animals in both groups. A significant (p < 0.05) increase in plasma testosterone concentration was observed in both groups after naloxone treatment, whereas, a decrease (p < 0.05) was observed in plasma prolactin levels after naloxone treatment. Thus, it can be concluded that endogenous opioids do play an important role in modulating plasma LH, prolactin and testosterone concentrations in male goats.

Keywords: Naloxone; Gonadotrophin; Prolactin; Testosterone; Male goat

1. Introduction

Endogenous opioid modulation of reproductive activity is well documented for female animals in several domesticated species (Whisnant et al., 1986; Gregg et al., 1986; Currie and Rawling, 1987; Byerley et al., 1992; Xia Orong, 1996). However, in the male animal of various species the information in this regard is sparse. In adult rats β-endorphins have been shown to inhibit testosterone secretion possibly because of its effect on synthesis of testosterone precursors (Chandershekar and Baskte, 1992). Earlier Cicero et al. (1989) reported an increase in serum LH and a dose independent increase in testosterone concentration in rats after naloxone treatment. Naloxone treatment was found to elevate plasma FSH levels but not plasma LH levels in immature pigs (Trudeau et al., 1989). Naloxone has been found to significantly
increase the release of LH and prolactin in stallions whereas, it has no effect in either plasma LH or prolactin levels in geldings (Aurich et al., 1996). In growing bull calves it has been indicated that the effect of naloxone on LH was age related, and a decrease of opioidergic inhibition of pulse frequency of LH at 12–18 weeks of age contributed to the overall increase in the circulating serum concentrations of LH (Evans et al., 1993). A significant correlation between basal testosterone levels and increase in LH in stallions has been reported in growing bull calves (Pluschke, 1994) indicating that opioid inhibition of LH release is related to the presence of gonadal steroids in this species. In male goats, (Fuentes et al., 1997, 1998) have reported an increase in libido and testosterone levels during non-breeding season. The caprine species has received little attention with respect to opioid modulation of reproductive or endocrine activity. The objective of the present study was to characterise the endocrine response of the male goat to naloxone.

2. Material and methods

Ten adult Beetal male goats aged 4–6 year were randomly selected from the University goat flock (45–60 kg body weight (BW)) and used in this study. The experiment was conducted during the month of May (non-breeding season) 1997. The goats were fed a legume hay grain diet and had free access to water. The bucks were divided randomly into two groups. The goats in Group I were given naloxone hydrochloride intravenous (Sigma, USA) at a rate of 1 mg/kg BW, while the bucks in the other group were given naloxone at a rate of 2 mg/kg BW as a bolus dose intravenously. An indwelling catheter was placed in the jugular vein of each buck 20 h prior to the onset of blood sampling. Blood samples were collected at 15 min intervals from 2 h before to 2 h after the naloxone treatment. The blood samples were stored in an ice bath prior to centrifugation at 3000 rpm in a refrigerated centrifuge (5°C). The plasma was separated within 30 min of sample collection and stored at −75°C, pending hormonal analysis.

The plasma LH, FSH and prolactin levels were quantified using the heterologous double antibody radioimmunoassay techniques standardised in the laboratory (Kaker et al., 1980; Kaker et al., 1982; Razdan et al., 1982). The sensitivities, intra-assay and inter-assay coefficients of variation were 0.125 ng/tube, 4.5 and 4.9% for plasma LH, 0.25 ng/tube, 9.3 and 10.2% for plasma FSH and 2.5 ng/tube, 7.6 and 9.4% for the prolactin assay systems, respectively.

Plasma testosterone concentrations were also determined by the direct solid phase 125I RIA kit (Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA).

The data obtained were analysed using a general linear model two way Manova (multivariate anova), treating dose and phase (pre-treatment and post-treatment) as fixed independent variables and hormone concentrations as dependent variables with sample sequences as a covariate using a statistical software package on a PC (SPSS version 7.51, release of 1996; SPSS; Chicago, USA).

3. Results

Plasma concentration of hormones (range and mean ± SEM) at two dose levels of naloxone treatment prior to and after administration of the opioid antagonist are presented in Table 1. The mean and SEM of the hormone levels at each 15 min sampling is set out in Fig. 1. It was evident from the statistical analysis that only in the case of plasma LH and testosterone the difference in the hormone concentrations at the two dose levels of naloxone were significant (p < 0.05). The testosterone levels started rising in both groups 15 min after the increased plasma LH concentration resulting from naloxone treatment. Thus, by only these two hormones exhibited naloxone dose related responses in their circulating plasma concentrations. Plasma LH was elevated by the higher (2 mg/kg BW) dose of naloxone as was the case with the gonadal hormone testosterone. The increase in plasma LH was significant (p < 0.05) and amounted to a 4-fold increase in level by 30 min after naloxone treatment, compared to the prenaloxone period concentrations. The increase was only 3-fold in the case of 1 mg/kg BW naloxone injection after 30 min. The circulating levels of FSH did not change as a result of naloxone administration (Fig. 1). It can be seen that plasma FSH was not affected by the treatment with naloxone. On the other hand, plasma LH and testosterone concentrations in the circulation was elevated and plasma prolactin concentrations decreased. The
Table 1
Plasma concentrations of LH, FSH, prolactin and testosterone before and after naloxone treatment in bucks

<table>
<thead>
<tr>
<th></th>
<th>Pre-naloxone(^a)</th>
<th>Post-naloxone(^a)</th>
<th>Pre-naloxone(^a)</th>
<th>Post-naloxone(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg/kg BW (Group I)</td>
<td>2 mg/kg BW (Group II)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>Range</td>
<td>0.17–0.66</td>
<td>0.49–1.87</td>
<td>0.26–0.64</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>0.41(^A) ± 0.03</td>
<td>0.91(^BC) ± 0.05</td>
<td>0.44(^A) ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>24.46(^A) ± 1.57</td>
<td>24.82(^A) ± 1.84</td>
<td>22.51(^A) ± 1.16</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>Range</td>
<td>83.94–143.02</td>
<td>50.29–155.07</td>
<td>88.76–167.61</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>117.42(^B) ± 4.98</td>
<td>90.52(^A) ± 7.08</td>
<td>124.80(^B) ± 5.64</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>Range</td>
<td>17.67–32.46</td>
<td>23.49–89.29</td>
<td>18.15–37.47</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>24.52(^A) ± 1.71</td>
<td>57.53(^BC) ± 2.94</td>
<td>25.64(^A) ± 1.74</td>
</tr>
</tbody>
</table>

\(^a\) Mean bearing dissimilar letters differ significantly from each other (\(p < 0.05\)).

Fig. 1. Effect of opioid antagonist naloxone on plasma (a) LH, (b) FSH, (c) prolactin and (d) testosterone in male goats. (zero on X-axis is the time of administration of naloxone.)
statistical analysis also indicated that the sampling sequence did not affect the hormone concentrations.

4. Discussion

The consistent increase in plasma LH immediately following naloxone injection in these bucks (Fig. 1, Table 1) demonstrated a considerable involvement of endogenous opioids in the modulation of plasma LH levels in the blood circulation. As naloxone is relatively specific as an endogenous opioid receptor antagonist (Goldstein, 1984), it can be concluded that endogenous opioids are inhibitory to LH release in male goats. The LH release by naloxone treatment due to disinhibition of the opioid effect has also been observed in female goats (Xia Orong, 1996). The plasma LH levels reported in this study are in agreement with other reports in steers (Peck et al., 1990) in rats (Cicero et al., 1989 and El-Sheltawi et al., 1995), in stallions (Aurich et al., 1996), in prepubertal bull calves (Rawlings et al., 1991 and Evans et al., 1993). However, Trudeau et al. (1989) and Prunier et al. (1990) did not find any increase in plasma LH secretion after naloxone injection in immature male pigs and suggested that endogenous opioids are not involved in regulation of LH in the immature male pig.

Plasma FSH concentrations have been reported to be increased by naloxone treatment in intact and castrated rats (El-Sheltawi et al., 1995), in young ram lamb (Rawlings et al., 1991) and in immature male pigs (Trudeau et al., 1989). In contrast to this study, naloxone administration did not increase plasma FSH concentration in Group I, however there was slight increase in FSH concentration in Group II, which was not significant. Lincoln (1988) reported no influence of naloxone on serum FSH secretion and Rawlings et al. (1993) suggested that in the adult ewe, naloxone does not appear to influence FSH secretion. This leads to the conclusion that the opioid antagonist acts directly on the LH producing cells at the hypothalamic level and is not affected by the GnRH releasing mechanism at the hypothalamic level. However, it cannot be assessed from this study as to whether the synthesis of LH or its release from hypophyseal cells or both are influenced by naloxone. It has been hypothesized from studies in rats (Miller et al., 1986) that the mechanism of action may be through interference with the GnRH pulse generator. However, lack of response in FSH and response only in plasma LH obtained in this study on bucks do not support this hypothesis.

Administration of naloxone decreased plasma prolactin concentrations in ewes (Gregg et al., 1986) and intact and castrated male rats (El-Sheltawi et al., 1995). Aurich et al. (1996) found no effect of naloxone on plasma prolactin levels in geldings, but increased prolactin concentrations after naloxone was reported in stallions by the same researchers. In the present study, plasma prolactin concentration decreased significantly ($p<0.05$) after naloxone injection in both treatment groups. A significant ($p<0.05$) increase in plasma testosterone levels as reported in this study, is in agreement with results reported earlier (Cicero et al., 1989; Pluschke, 1994; Fuentes et al., 1997; Fuentes et al., 1998). The effect of naloxone on plasma testosterone levels can be attributed to the after effect of elevated LH levels. Similar conclusion was reported by Miller et al. (1986). Naloxone probably acts by blocking the inhibitory effect on testosterone precursor synthesis by the endogenous opioids, as is reported to occur in rats (Chandershekar and Baskte, 1992).

It was evident that endogenous opioids do play an important role in modulating LH, prolactin and testosterone concentrations in bucks of the Indian sub-tropical breed of *Beetal* goats. There is also an indication that at least in this species, naloxone has acted independently of its antagonistic effect on the action of endogenous opioid peptides on GnRH pulse generator.

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References


