GnRH in the male dog: dose–response relationships with LH and testosterone

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Dose–response relationships between GnRH and LH, and between GnRH and testosterone, were investigated in six male dogs by intravenous administration of a GnRH analogue at different doses. Each dose of GnRH analogue induced an immediate rise in the plasma concentration of LH and then a rise in plasma testosterone concentration. Irrespective of the dose used, the rise in testosterone began 10 min after the GnRH injection. Administration of GnRH at doses of 0.01, 0.1, 1, 10 and 100 $\mu\text{g kg}^{-1}$ resulted in maximum LH concentrations in plasma (mean $\pm$ SEM; $n = 6$) of 22 ± 7 , 27 ± 6 , 40 ± 7 , 57 ± 13 and $56 \pm 10 \mu\text{g l}^{-1}$, respectively. These doses induced maximum concentrations of testosterone in plasma (mean $\pm$ SEM; $n = 6$) of 16 ± 4 , 20 ± 4 , 22 ± 3 , 22 ± 4 and $24 \pm 7 \text{ nmol l}^{-1}$, respectively. The lag time between peak concentrations of LH and testosterone varied from 35 to 55 min. The calculated maximum response of testosterone to LH, secreted by the anterior pituitary after GnRH injection, was 1.8 times higher than to GnRH. It was concluded that intravenous administration of GnRH induced marked and dose-dependent increases in plasma concentrations of LH and testosterone, and that there does not appear to be a direct effect of GnRH on Leydig cells in male dogs.

Introduction

The hypothalamus–pituitary–adrenocortical (HPA) axis has been studied extensively in dogs (Kempainen *et al.*, 1986; Middleton *et al.*, 1987; Mol and Rijnberk, 1989). Data on the hypothalamus–pituitary–testis (HPT) axis in the dog, however, are scarce (Falvo and Vincent, 1980; Falvo *et al.*, 1980). In male dogs a linear trend in LH responses was observed after intravenous administration of GnRH at doses varying from 0.005 to 0.25 $\mu\text{g kg}^{-1}$ (Falvo *et al.*, 1982), but, to our knowledge, there are no reports on dose–response relationships between GnRH and LH and testosterone in males of any species.

The present study was undertaken to characterize pituitary and testicular responses to intravenous administration of a GnRH analogue in dogs.

Materials and Methods

Animals

Six healthy adult male beagles, 6.3 ± 2.6 (mean $\pm$ SD) years of age and weighing $14.5 \pm 2.3 \text{ kg}$, were used in this study. Palpation of the testes of each dog revealed no abnormalities.

The animals were housed individually in indoor kennels with access to separate outdoor runs for about 2 h per day, and were exposed to a normal daylight regimen (the experiment was performed in September and October). The dogs were fed a standard commercial diet in the morning and water was always available. Because their fertility was studied simultaneously, the dogs were accustomed to twice weekly semen collection by manual manipulation in the presence of a bitch in oestrus. Intervals between GnRH administration and semen collection, and vice versa, were at least 24 h.

Experimental design

Each dog was treated with a GnRH analogue (Fertagyl: Intervet International BV, Boxmeer) containing 0.1 mg gonadorelin ml^{-1} . Six doses (0, 0.01, 0.1, 1, 10 and 100 $\mu\text{g kg}^{-1}$ body weight) were used in a 6×6 Latin square design (Spriet and Simon, 1985). All dogs were injected via the cephalic vein with either GnRH diluted in a solution of 9 g NaCl l^{-1} or an equal volume of diluent only (controls). Treatments were given at weekly intervals.

Starting at 08:00 h, blood samples were collected at -20 , -10 , 0, 5, 10 and at 10 min intervals until 160 min after injection of GnRH. Blood was collected by jugular venepuncture into sterile 5 ml heparinized tubes (Vacutainer; Becton-Dickinson, Etten-Leur), which were immediately placed on ice until centrifugation (10 min, 1600 g) at 4°C within 30 min. Plasma was stored at -25°C until assayed.

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Hormone determinations

Plasma LH concentrations were assessed by a heterologous radioimmunoassay as described by Nett *et al.* (1975). The sheep antibody, CSU-204 (kindly supplied by G.D. Niswender, Colorado State University), radioiodinated NIAMDD-bLH-4 and canine pituitary standard LER 1685-1 were used in this assay.

Plasma testosterone concentrations were measured by radioimmunoassay after extraction according to the method of Dieleman *et al.* (1983). Validation of this RIA method in dog plasma produced intra- and interassay coefficients of variation of 12 and 14%, respectively; the sensitivity was 0.35 nmol l^{-1} .

Statistical analysis and calculations

Pretreatment plasma values were calculated separately for each dose of GnRH, except the control dose, by averaging the plasma values obtained at -20, -10 and 0 min in the six dogs. Each of the five pretreatment values was tested with a paired *t* test against the corresponding plasma value at 160 min to determine whether plasma hormone concentrations had returned to baseline values within 160 min after injection.

A response was defined as the total area under the curve corresponding to a certain dose of GnRH. This area was calculated geometrically by computer for each response of LH or testosterone from the time of GnRH injection until 160 min thereafter, and was corrected for the area under the control curve. Dose-response relationships were calculated on the basis of reciprocal response versus reciprocal dose according to the method of least squares regression (Snedecor and Cochran, 1980). The maximum response was derived from the intercept of the regression equation. Coefficients of correlation were tested for significance by a one-tail test. The level of significance was $P \leq 0.05$.

Results

No adverse effects of GnRH administration on health or behaviour were observed in any of the dogs. Pretreatment plasma concentrations (mean $\pm$ SEM; $n = 30$) were $4.6 \pm 0.5 \mu\text{g l}^{-1}$ for LH and $9.7 \pm 1.7 \text{ nmol l}^{-1}$ for testosterone. Plasma concentrations (mean $\pm$ SEM; $n = 30$) determined at 160 min were $3.9 \pm 0.4 \mu\text{g l}^{-1}$ for LH and $7.1 \pm 1.3 \text{ nmol l}^{-1}$ for testosterone (Fig. 1). There were no significant differences in LH or testosterone between the mean pretreatment value and mean value obtained at 160 min, nor among the five pretreatment values, nor among the five values obtained at 160 min.

Each GnRH dose induced an immediate increase in the plasma concentration of LH and then an increase in concentration of testosterone in plasma (Fig. 1). Irrespective of dose, the increase in testosterone began 10 min after GnRH injection. Administration of GnRH at doses of 0.01, 0.1 and $1 \mu\text{g kg}^{-1}$ resulted in maximum concentrations of LH in plasma (mean $\pm$ SEM; $n = 6$) at 5 min after injection of 22 ± 7 , 27 ± 6 and $40 \pm 7 \mu\text{g l}^{-1}$, respectively. The GnRH doses of 10 and $100 \mu\text{g kg}^{-1}$ resulted in mean maximum LH concentrations at 10 and 20 min after injection of 57 ± 13 and $56 \pm 10 \mu\text{g l}^{-1}$, respectively. Maximum plasma testosterone concentrations (mean $\pm$ SEM; $n = 6$) induced by GnRH doses

Fig. 1. Mean responses of (a) LH and (b) testosterone to six doses of a GnRH analogue in six male dogs; (○) controls, (●) $0.01 \mu\text{g kg}^{-1}$, (□) $0.1 \mu\text{g kg}^{-1}$, (■) $1 \mu\text{g kg}^{-1}$, (△) $10 \mu\text{g kg}^{-1}$, (▲) $100 \mu\text{g kg}^{-1}$.

of 0.01 and $0.1 \mu\text{g kg}^{-1}$ at 40 min after injection were 16 ± 4 and $20 \pm 4 \text{ nmol l}^{-1}$, respectively. The GnRH doses of 1, 10 and $100 \mu\text{g kg}^{-1}$ resulted in mean maximum concentrations of testosterone in plasma at 60 min after injection of 22 ± 3 , 22 ± 4 , and $24 \pm 7 \text{ nmol l}^{-1}$, respectively. The lag time between LH and testosterone peaks varied from 35 to 55 min.

The responses of LH and testosterone to GnRH administration are shown as a function of the logarithm of the GnRH dose (Fig. 2). The corresponding regression equations for reciprocal dose versus reciprocal response were $Y \times 10^4 = 0.15X + 8.10$ ($r = 0.80$; $P = 0.05$; $n = 5$) and $Y \times 10^4 = 0.05X + 7.50$ ($r = 0.64$; $n = 5$), respectively. Similarly, the reciprocal dose-response relationship between LH and testosterone was $Y = 0.38X + 0.00042$ ($r = 0.97$; $P < 0.005$; $n = 5$). The calculated maximum response of testosterone to LH, secreted by the anterior pituitary after GnRH injection, was 1.8 times higher than to GnRH.

Discussion

In this experiment in male dogs, single intravenous injections of a GnRH analogue resulted in a dose-dependent increase in

Fig. 2. Responses (mean ± SEM) of (a) LH and (b) testosterone to intravenous administration of five doses of a GnRH analogue in six male dogs.

plasma concentrations of both LH and testosterone. A dose of 0.01 µg GnRH kg⁻¹ induced an LH response of 22 µg l⁻¹. This response is similar to that assessed in another study in male dogs (Falvo *et al.*, 1980), in which intravenous administration of 0.25 µg GnRH kg⁻¹ resulted in an LH response of 21 µg l⁻¹.

Significant relationships were found between GnRH dose and LH response as well as between LH dose, being the LH response to GnRH, and testosterone response, but not between GnRH dose and testosterone response. These findings suggest that Leydig cells were activated by LH released after GnRH injection rather than by the GnRH injected. This is substantiated by the fact that the calculated maximum response of

testosterone to LH was 1.8 times higher than the calculated maximum response of testosterone to GnRH. Further calculations show that the maximum response of LH to GnRH is 0.12 × 10⁴ (min × µg l⁻¹). Substitution of this value in the dose-response relationship of LH and testosterone produces a response of 0.13 × 10⁴ (min × µg l⁻¹), which equals the maximum response of testosterone to GnRH. It can therefore be concluded that direct effects of GnRH on Leydig cells which have been documented in rats (Clayton *et al.*, 1985) appear to be absent in dogs as they are in rams (Meijer *et al.*, 1989).

In summary, we found that intravenous administration of a GnRH agonist induced distinct and dose-dependent increases in plasma LH as well as testosterone concentrations, and that there appears to be no direct effect of GnRH on Leydig cells in male dogs.

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