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 µg kg⁻¹ resulted in maximum LH concentrations in plasma (mean ± SEM; n = 6) of 22 ± 7, 27 ± 6, 40 ± 7, 57 ± 13 and 56 ± 10 µU l⁻¹, respectively. These doses induced maximum concentrations of testosterone in plasma (mean ± SEM; n = 6) of 16 ± 4, 20 ± 4, 22 ± 3, 22 ± 4 and 24 ± 7 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 (Kemppainen et al., 1986; Middleton et al., 1987; Mol and Rijnberk, 1989). Data on the hypothalamus–pituitary–testes (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 µg 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 ± SD) years of age and weighing 14.5 ± 2.3 kg, were used in this study. Palpation of the testes of each dog revealed no abnormalities.

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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), radiiodinated 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 Dielemann 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 ± SEM; \( n = 30 \)) were 4.6 ± 0.3 pg l\(^{-1}\) for LH and 9.7 ± 1.7 nmol l\(^{-1}\) for testosterone. Plasma concentrations (mean ± SEM; \( n = 30 \)) determined at 160 min were 3.9 ± 0.4 pg l\(^{-1}\) for LH and 7.1 ± 1.3 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 pg kg\(^{-1}\) resulted in maximum concentrations of LH in plasma (mean ± SEM; \( n = 6 \)) at 5 min after injection of 22 ± 7, 27 ± 6 and 40 ± 7 pg l\(^{-1}\), respectively. The GnRH doses of 10 and 100 pg kg\(^{-1}\) resulted in maximum LH concentrations at 10 and 20 min after injection of 57 ± 13 and 56 ± 10 pg l\(^{-1}\), respectively. Maximum plasma testosterone concentrations (mean ± SEM; \( n = 6 \)) induced by GnRH doses of 0.01 and 0.1 pg kg\(^{-1}\) at 40 min after injection were 16 ± 4 and 20 ± 4 nmol l\(^{-1}\), respectively. The GnRH doses of 1, 10 and 100 pg kg\(^{-1}\) resulted in mean maximum concentrations of testosterone in plasma at 60 min after injection of 22 ± 3, 22 ± 4, and 24 ± 7 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 \( Y \times 10^x = 0.15x + 8.10 \) (\( r = 0.80; \ P = 0.05; \ n = 5 \)) and \( Y \times 10^x = 0.05x + 7.50 \) (\( r = 0.64; \ n = 5 \)), 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
plasma injection response 0.25 dogs response male intravenous Fig. tiated that GnRH Significant LH (Falvo et al., 1985) responses to LH in dogs. 

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.

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 $0.12 \times 10^3$ (min × µg l$^{-1}$). Substitution of this value in the dose–response relationship of LH and testosterone produces a response of $0.13 \times 10^3$ (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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References

Clayton RN, Detta A, Nikula H and Huhtaniemi IT (1985) Physiological role of putative testicular gonadotrophin releasing hormone (GnRH) Medical Biological 63 201–209

Dieleman SJ, Kruip ThAM, Fontijne P, De Jong WHR and Van der Weyden GC (1983) Changes in oestradiol, progesterone, and testosterone concentrations in follicular fluid and in the micromorphology of proovulatory bovine follicles relative to the peak of luteinizing hormone Journal of Endocrinology 97 31–42

Falvo RE and Vincent DL (1980) Testosterone regulation of follicle-stimulating hormone secretion in the male dog Journal of Andrology 1 197–201


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