PRELIMINARY INVESTIGATIONS OF THE EFFECT OF DIFFERENT DOSES GNRH (GONODDOTROPHIN RELEAZING HORMON) AND BOVINE LH (LISTERIZING HORMON) ON PLASMA LH AND TESTOSTERONE CONCENTRATIONS IN MALE DOGS

PENELITIAN PENDAHULUAN PENGARUH PEMBERIAN BERBAGAI DOSIS GNRH DAN LH SAPI TERHADAP KONSENTRASI PLASMA LH DAN TESTOSTERON PADA ANJING JANTAN

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ABSTRACT

Six male adult dogs were randomized into 2 equal groups. Group 1, dogs were injected intravenously with dose (GnRH) and their responses to testosterone and dose (LH) were measured at 10 minutes intervals. Group 2, dogs were injected intravenously with bovine LH and their responses to testosterone were measured at the same intervals as group 1. A single intravenous injection of GnRH resulted in increased plasma LH and testosterone concentration in all dose tested, while either doses of 0.2 μg/kg and 0.5 μg/kg of bovine LH could be used effectively to increased testosterone concentration in the male dogs. It can be concluded that bovine LH and GnRH chalengge test could be used to measure the pituitary sensitivity in male dogs.

Key words: LH, GnRH, testosterone and pituitary

ABSTRAK

Enam ekor anjing jantan dewasa dibagi rata secara acak menjadi dua kelompok yang sama. Kelompok 1, anjing diinjeksi secara intravena dengan dosis (GnRH) dan respon terhadap testosteron dan dosis (LH) diukur setiap interval 10 menit. Kelompok 2, anjing diinjeksi secara intravena dengan LH sapi dan respon terhadap testosteron diukur dengan interval yang sama seperti pada kelompok 1. injeksi tunggal GnRH secara intravena menyebabkan peningkatan konsentrasi plasma testosteron dan LH pada semua dosis yang diuji, sedangkan dosis LH sapi 0.2 μg/kg dan 0.5 μg/kg dapat digunakan secara efektif untuk meningkatkan konsentrasi testosteron pada anijng jantan. Dari penelitian ini dapat disimpulkan bahwa uji tantangan dengan LH sapi dan GnRH dapat digunakan untuk mengukur sensitifitas pituitari pada anjing jantan.

Kata kunci: LH, GnRH, testosteron dan pituitari

INTRODUCTION

The dose-response relationships between GnRH and LH, and between GnRH and testosterone has been invenstigated in six male dogs by intravenous administration of GnRH analogue (Fertagyl, intervet) at doses varying from 0.01 to 100 μg/kg (Knol *et.al.*, 1993). These workers found that each dose of GnRH analogue induced an acute rise in the plasma concentrations of LH, followed bay acute rice in plasma testosterone concentration. There are no reports on dose-response relationship between LH and testosterone when LH is injected into male dogs.

Table 1. Hormonal responses to GnRH challenge in male dofs (mean + SEM)

Dose of GnRH (μg/kg bw)	LH (ng/ml)			Testosterone (ng/ml)		
	Baseline level	Peak level	Return to baseline	Baseline level	Peak level	Return to baseline
2.5	0.9±0.3	2.2±0.6[a]	0.6±0.1	1.5±0.75	2.9±0.8[a]	1.0±0.5
5	0.4±0.9	2.0±0.8[a]	0.2±0.5	1.5±0.4	3.9±1.2[b]	1.4±0.6

Means with different superscript in the same columns are significantly different (P<0.05)

The present study was undertaken to characterise pituitary and testicular responses to intravenous administrations of different doses of a GnRH analogue and bovine LH. The dose rates of both hormones which stimulated the optimal response in male dogs was determined.

MATERIAL AND METHODS

Animals

Six male adult dogs ranging in age from 2 to 3 years and weighing about 8 to 15 kg were used in this study. They were assigned to 2 equal groups. Group 1 (n=3), each dog was injected i.v. with 2.5 μg/kg and 2 weeks later, with 5 μg/kg body weight of GnRH analogue (Fertagyl, Intervet). Groups 2 (n=3), each dog was injected with 0.2 μg/kg and, 2 weeks later, with 0.5 μg/kg of body weight of bovine LH.

Exogenous GnRH and bovine LH

GnRH analogue (Fertagyl, Intervet) and bovine LH (prepared by Peter Stanton, Prince Henry's Institute of Medical Research, Clayton) were used in this study. Plasma LH and testosterone responses were determined by taking blood samples for hormone assay at 10 minutes intervals after administering i.v. 2.5 μg/kg and 5 μg/kg body weight of GnRH and 0.2 μg/kg and 0.5 μg/kg body weight of bovine LH.

Hormone Assay

The concentration of plasma LH and testosterone were determined from blood samples collected intensively in every challenge test. Blood samples were taken at -40, -20, -10, 0 minutes and then at 10 minutes interval for 90 minutes, then 20 minutes intervals for 1 hour, to determine the acute release of LH and testosterone. Baseline levels were the mean of samples taken in the hour before challenge test. Peak levels were the mean of samples taken every 10 minutes for one hour after challenge test and return to baseline period are the means of samples taken every 20 minutes for one hour after 90 minutes of the challenge test. The procedure used for hormone assays were described previously Junaidi et. Al. (2000). The limit of detection was 0.6 + 0.2 ng/ml. The NSB was 5.3 + 1.7% of total counts. Included in each assay were six replicates of three pooled plasma samples containing 0.63 ng/ml, 1.76 ng/ml, and 4.47 ng/ml. They were used to estimat the coefficients of variation within assays (21.7 ± 1.07%, 11.0 + 1.6%, and 13.6 + 2.8%) and between assays (20.4%, 10.1%, and 10.6%).

Table 2. Testosterone response to bovine LH challenge in male dofs (n=3; mean + SEM)

Dose of bovine LH (μg/kg bw)	Testosterone (ng/ml)		
	Baseline level	Peak level	Return to baseline
0.2	1.3±0.3	2.4±0.4[a]	0.7±0.6[a]
0.5	1.3±0.4	3.2±0.6[b]	1.6±0.5[b]

Means with different superscript in the same columns are significantly different (P<0.05)

Statistical analyses

Hormone concentrations of testosterone and LH are shown as means + SEM. Differences between LH and testosterone concentrations between treatments were evaluated by ANOVA, followed by pairwise

comparisons of means TUKEY'S (HSD) using Statistic version 4.1 (c 1994, Analytical Software). The level of significant was set at P<0.005.

RESULT AND DISCUSSION

Release of LH and testosterone in response to exogenous GnRH

The patterns of LH and testosterone release after intravenous administration of 2.5 μg/kg of body weight of GnRH are shown in Fig. 1. The responses of

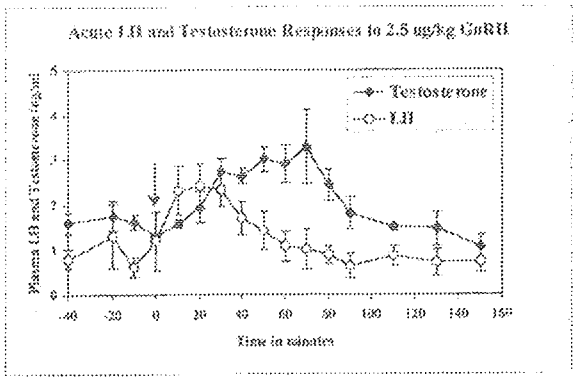

Fig. 1. Mean (± SEM) response of LH and testosterone to 2.5 μg/kg of body weight of GnRH in male dogs (n = 3).

LH and testosterone concentrations to 5 μg/kg of body weight of GnRH are depicted in Fig. 2. The summary data together with statistical analyses are included in Table 1.

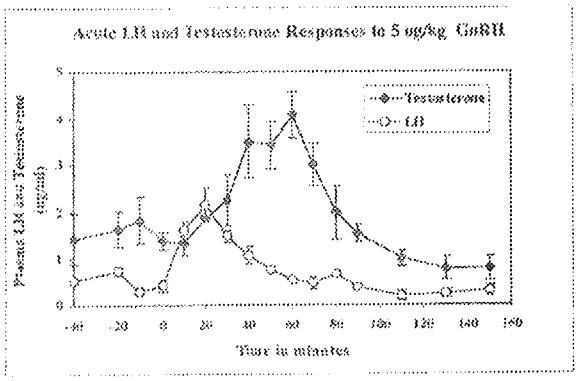

Fig. 2. Mean (± SEM) response of LH and testosterone to 5μg/kg of body weight of GnRH into male dogs (n = 5).

An LH peak occurred 20 minutes after i.v. injection of GnRH (Fig. 1), and was followed by testosterone peaked 40 minutes later. There was no significant (P<0.05) difference between peak level of LH at the dose of 2.5 μg/kg and 5 μg/kg. However, there was a significantly different (P>0.05) in the peak level of testosterone (2.9 + 0.8 ng/ml versus 3.9 + 1.2 ng/ml) (Table 1.)

Release of LH and testosterone in response to bovine LH

Changes in plasma concentrations of testosterone after i.v. injection with 0.2 μg/kg and 0.5 μg/kg of body weight of bovine LH are shown in Fig. 3 and 4, respectively. Summary data, together with statistical analyses, are included in Table 2.

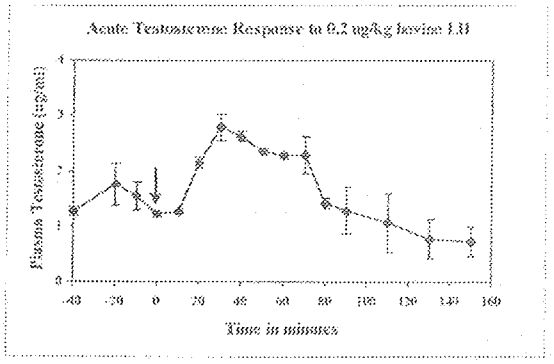

Fig. 3. Mean (±SEM) response of testosterone to 0.2 μg/kg of body weight of bovine LH in male dogs (n = 3).

A testosterone peak occurred 40 minutes after i.v. injection of both doses tested. There was significantly different (P>0.05) between peak level of testosterone on the dose of 2.5 μg/kg and 0.5 μg/kg (2.4+0.4 ng/ml versus 3.1+0.6 ng/ml) (Table 2).

DISCUSSION

In this experiment, a single intravenous injection of a GnRH analogue resulted increased plasma LH and testosterone concentrations in male dogs in all dose tested. The concentrations of plasma

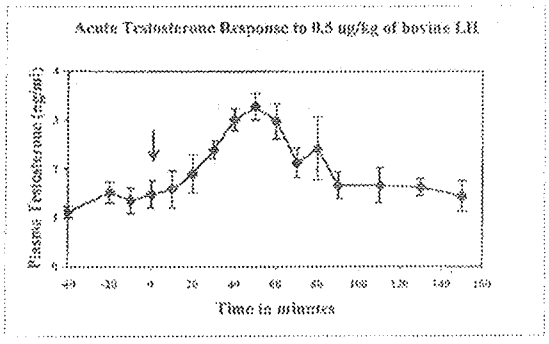

Fig. 4. Mean (± SEM) response of testosterone to 5μg/kg of body weight of GnRH into male dogs (n = 3).

LH reached a maximum within 20 minute of the injection, while the testosterone concentrations reached maximum 40 minutes later. This response is similar to that assessed in another study in male dogs (Jones et al., 1976), in which intravenous administrations of 5 μg GnRH resulted in maximal LH response at 15 minutes and a maximal testosterone concentration reached at 40

minutes. This lag time between peak concentrations of LH and testosterone was in agreement with previous report that peak testosterone values occure 15 to 105 minutes after the LH peak (Guenzel-Apel et al., 1994).

There was significantly difference (P>0.05) between the peak testosterone concentrations in response to bovine LH at dose rates of 0.2 μg/kg BW (2.4+0.4) and 0.5 μg/kg BW (3.2+0.6). this finding was in agreement with those reported by Fraser and Lincoln (1980) in rams. Lincoln et al., (1986) reported that the injection of a physiological dose of GnRH was stimulate the release of LH.

In this study, we found that intravenous administration of a 5 μg/kg of body weight of GnRH increases plasma LH concentration to a suitable concentration for use in the challenge trials, while either doses of 0.2 μg/kg and 0.5 μg/kg of bovine LH could be used effectively to increase testosterone concentrations in the male dogs. It can be suggested that the LH response to a GnRH challenge test could be used to measure the function of pituitary in male dogs.

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