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Short Communication

A Single Administration of the GnRH Antagonist Acyline Inhibits Basal and GnRH-Stimulated Serum Testosterone Concentrations in Male Dogs

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Contents

The objective of this study was to describe testosterone (T) response to GnRH challenge in antagonist-treated dogs over a 30-day period. Eight mongrel dogs were randomly assigned to either the GnRH antagonist acyline 330 μg/kg sc (ACY; n = 4) or a placebo group (PLA; n = 4). The dogs were serially challenged with the GnRH agonist, buserelin 0.2 μg/kg sc on days −1, 1, 3, 7, 10, 14, 21 and 30. On these days, blood samples for T determinations were collected before (−30 min) and 60, 120 and 180 min after the agonist injection. Basal (−30 min) and post-GnRH agonist stimulation T values were compared by ANOVA for repeated measures. Before treatments (day −1), there were no differences in basal T serum concentrations between groups ($p > 0.1$). After treatments, basal T showed a significant interaction between treatment and day ($p < 0.05$). Furthermore, when both groups were analysed independently, basal T varied in the ACY ($p < 0.01$) but not in the PLA group ($p > 0.1$). On day −1, before treatments, the stimulation tests had only a time effect ($p = 0.05$) although on days 1 ($p < 0.01$), 3 ($p < 0.01$), 7 ($p < 0.01$), 10 ($p < 0.01$) and 14 ($p < 0.05$), the response to the agonist differed between groups, becoming similar on days 21 ($p > 0.05$) and 30 ($p > 0.05$). It was concluded that, in dogs, a single administration of the GnRH antagonist prevented canine gonadal axis to physiologically respond to agonistic challenge during 14 days.

Introduction

Gonadotropin-releasing hormone (GnRH) antagonists bind to gonadotrope GnRH receptors and compete successfully with endogenous GnRH molecules for specific membrane receptor occupancy (Vickery 1985). This leads to an immediate dose-related arrest of gonadotropin secretion without the initial 'flare effect' that characterizes agonistic effect (Heber et al. 1982).

Several antagonists of GnRH have shown to suppress luteinizing hormone (LH) and testosterone (T) secretion in domestic and laboratory animals (Vickery et al. 1984; Mann et al. 1987) and humans (Bremner et al. 1991). Therefore, they are being considered as possible treatments of hormone-dependent diseases and male contraceptives.

The first-generation GnRH antagonists had a limited duration of action, and therefore, they had to be administered daily to be bioactive. Large doses were also required to obtain adequate suppression of the GnRH receptor (Vickery 1985). They were hydrophobic with solubility limitations inducing nodule formation at the site of injection. They also had a tendency to produce allergic local and systemic side effects (Vickery 1985). Those problems were overcome with the new third-generation antagonists like teverelix, abarelix, cetrorelix, ganirelix and acyline, which are well tolerated. Particularly, acyline [Ac-D2Nal-D4Cpa-D3Pal-Ser-4Aph(Ac)-D4Aph(Ac)-Leu-ILys-Pro-DAla-NH2] is more potent and of longer duration than other third-generation antagonists (Herbst et al. 2004).

In domestic dogs, new GnRH antagonists efficiently and safely controlled different stages of reproduction (Gobello 2007). In bitches, acyline interrupted oestrous cycle and gestation and blocked ovulation (Valiente et al. 2009a,b). In male dogs, a single administration of the same antagonist reversibly impaired semen quality (Valiente et al. 2007) and decreased basal serum gonadotropins and T concentrations during 10 days (García Romero et al. 2009). A detailed description of the gonadal axis functionality under antagonistic blockade will further clarify effect of this new GnRH antagonist on canine species. For this reason, the objective of this study was to describe T response to GnRH challenge in antagonist-treated dogs over a 30-day period.

Materials and Methods

Animals

Eight reproductively normal (assessed by physical and seminal evaluation) mongrel dogs, 2–6 years old, body weight 12–35 kg, were included in this study. The dogs were kept under natural photoperiod in outside–inside kennels, fed commercial dog food and given free access to water.

On day 0, all the animals were randomly assigned to either the GnRH antagonist acyline (Contraception & Reproductive Health Branch Center for Population Research, NIH, Bethesda, MD, USA) 330 μg/kg sc (ACY; n = 4) or a placebo group receiving the corresponding equal volume of physiological solution sc (PLA; n = 4). Acyline was provided in a lyophilized powder that was suspended in sterile distilled water (concentration, 2 mg/ml). The dose used was based on previous studies (Valiente et al. 2007; García Romero et al. 2009). This investigation was approved by the institutional ethics committee.

Blood sampling, challenge tests and hormone determinations

The dogs were serially challenged with the GnRH agonist, buserelin (Pyr-His-Trp-Ser-Tyr-D-Ser(Bu^t)-Leu-Arg-Pro

Ethylamide; Receptal®; Intervet, Buenos Aires, Argentina) 0.2 μg/kg sc on days −1, 1, 3, 7, 10, 14, 21 and 30. On these days, blood samples for T determinations were collected before (−30 min) and 60, 120 and 180 min after the agonist injection. Agonist challenges were performed between 09:00 and 11:00 am. Samples were centrifuged for 15 mins (3000 × ***g***), serum obtained and stored at −20°C until hormone assays. Serum T was measured by RIA, using a solid-phase kit (Coat-A-Count; DPC1, Los Angeles, CA, USA). The kit sensitivity was 0.04 ng/dl. All T samples were determined in the same assay. For the hormone assays, intra- and inter-assay CVs were < 10%.

Statistical analysis

Basal (−30 min) and post-GnRH agonist stimulation T values were compared by ANOVA for repeated measures, followed by Tukey's comparison test. Testosterone concentrations were expressed as mean ± SEM and the level of significance set at $p \leq 0.05$ (SIGMA STAT; SPSS Inc., Chicago, IL, USA).

Results

Before treatments (day −1), there were no differences in basal T (−30 min) serum concentrations between groups ($p > 0.1$). After treatments, basal T showed a significant interaction between treatment and day ($p < 0.05$). Furthermore, when both groups were analysed independently, basal T varied in the ACY ($p < 0.01$) but not in the PLA group ($p > 0.1$; Fig. 1).

On day −1, before treatments, the stimulation tests had only a time effect ($p = 0.05$) although on days 1 ($p < 0.01$), 3 ($p < 0.01$), 7 ($p < 0.01$), 10 ($p < 0.01$) and 14 ($p < 0.05$), the response to the agonist differed between groups, becoming similar on days 21 ($p > 0.05$) and 30 ($p > 0.05$; Fig. 2).

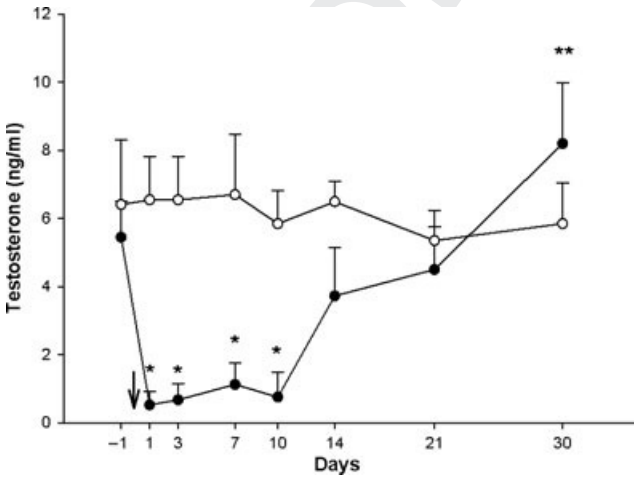

Fig. 1. Basal serum testosterone concentrations (mean ± SEM) of male dogs administered either (○) physiological solution sc (PLA = 4) or (●) acyline (330 μg/kg sc; ACY = 4) on day 0 (arrow). Asterisks represent values that differ at $p < 0.01$

Discussion

In male mammals, basal T serum concentrations are not sufficiently informative, and challenge tests are frequently necessary for both clinical and research purposes. Up to the authors' knowledge, this is the first serial evaluation of canine gonadal axis functionality under a GnRH antagonist treatment. The administration of GnRH agonists has been useful for evaluating pituitary-gonadal function in many species (Chakraborty et al. 1979; Brown et al. 1988) including dogs (Shille and Olson 1989), and response differences have been found among them (Jimenez Severiano et al. 2007).

In line with our previous work (García Romero et al. 2009), in the present study, a single administration of the potent, third-generation GnRH antagonist acyline decreased serum basal T concentrations during 10 days. An increased basal T value was also observed at the end of the experiment (day 30) possibly because of a rebound hyperstimulation effect. In normal men, a rebound increase in gonadotropins to concentrations exceeding baseline has been shown after cessation of GnRH antagonist treatment (Behre et al. 1997). A similar response has also been described in rams previously treated with GnRH antagonists (Jimenez Severiano et al. 2007). These rebound effects are probably caused by an increased secretion of native GnRH from the hypothalamus as a result of diminished negative feedback suppression by lowered T or interrupted negative short-loop feedback regulation of GnRH by the antagonist.

As expected, before the initial treatments (day −1), no significant differences were found in the stimulation tests of both groups, which presented a normal increasing T pattern after challenge. Conversely, from day 1 to 7, the response to the GnRH agonist was completely blunted in the acyline but not in the placebo group. On days 10 and 14, a progressive tendency to recover a physiological response began to be visible in the acyline group, although the results go on being significantly different between groups. The present hormonal outcome explains the decrease in ejaculate volume, sperm concentration and sperm motility reported in a previous clinical canine study in which the same antagonist and dose was used (Valiente et al. 2007). A loss in responsiveness to challenge with exogenous GnRH after antagonist treatments has also been demonstrated in rams and bulls, being greater in the latter (Jimenez Severiano et al. 2007).

Although from the third week after treatment on the response to stimulation was not statistically different between groups, on day 21, T increase was poor in the antagonist-treated dogs. This latter suggests a remnant hypofunctionality of the axis that could have been not statistically evidenced because of the limited number of animals used. At the end of the study (day 30), when basal T was elevated in acyline group, no further increase could be produced after buserelin. It was concluded that, in dogs, a single administration of the GnRH antagonist prevented canine gonadal axis to physiologically respond to agonistic challenge during 14 days. Further work on new GnRH antagonists in male dog is warranted before they could be widely indicated for clinical, biotechnological and contraceptive purposes in this species.

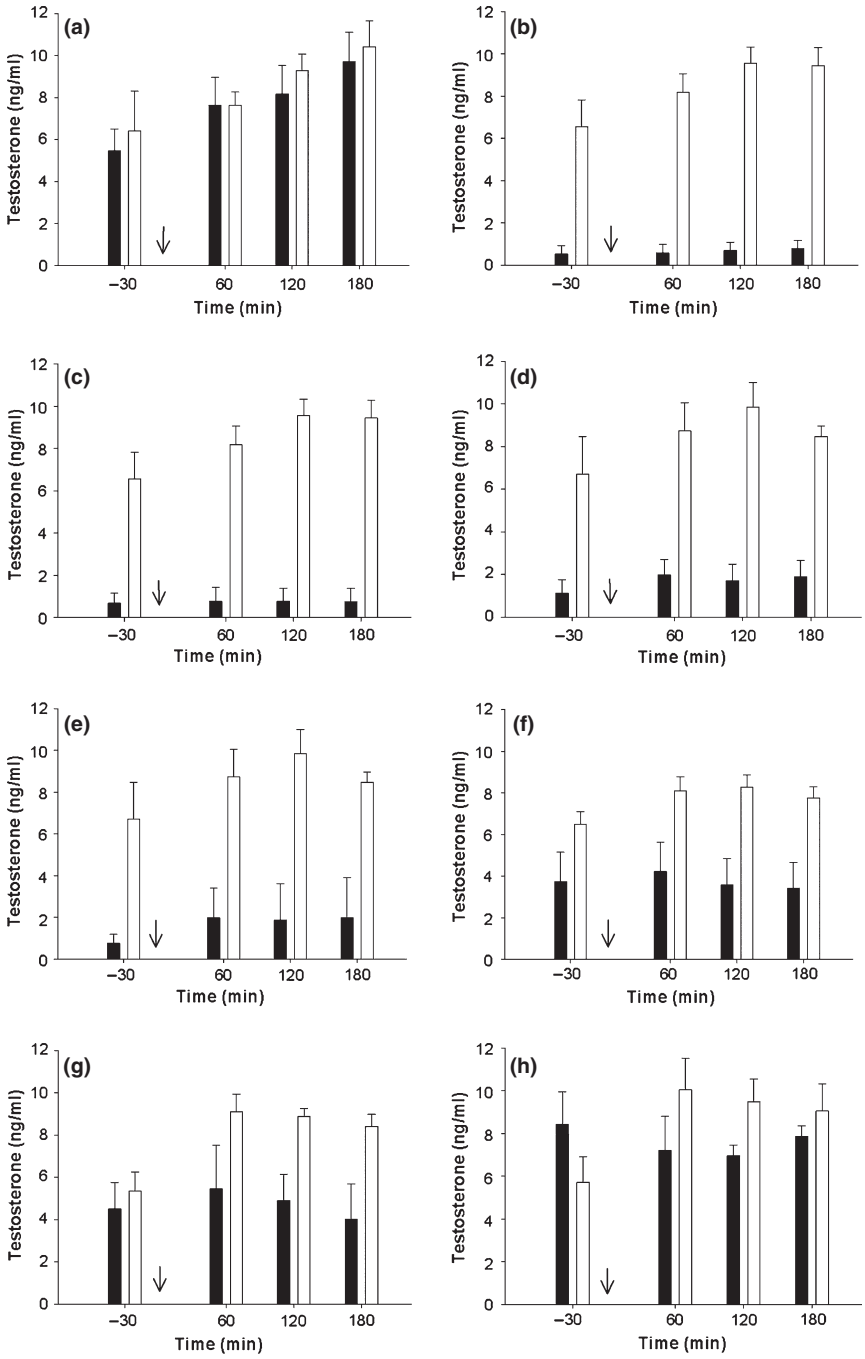

Fig. 2. Serum testosterone concentrations (mean ± SEM) of the same dogs and experiment of Fig. 1 30, 60, 120 and 180 min before [day −1 (a; $p > 0.1$)], and after [day 1; (b; $p < 0.01$), day 3 (c; $p < 0.01$), day 7 (d; $p < 0.01$), day 10 (e; $p < 0.01$), day 14 (f; $p < 0.05$), day 21 (g; $p > 0.05$) and day 30 (h; $p > 0.05$)] buserelin challenge (0.2 μg/kg sc; arrows). Black and white bars represent PLA and ACY groups, respectively

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Author contributions

All authors participated equally in the designed study, analysed data and drafted paper.

Conflict of interest

None of the authors have any conflict of interest to declare.

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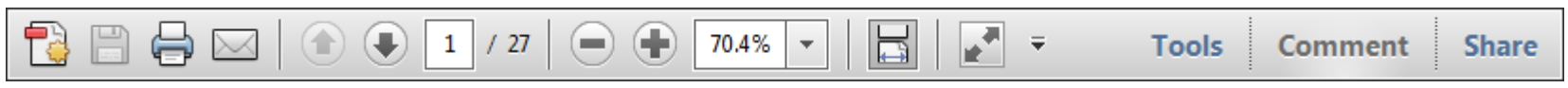

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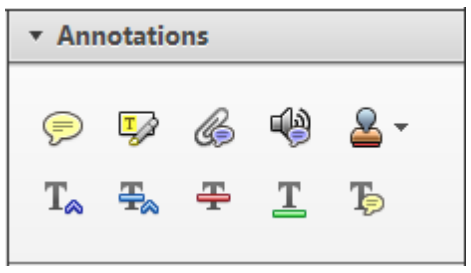

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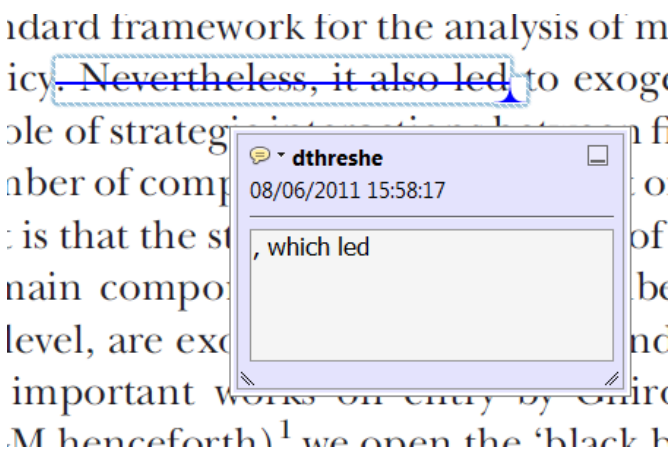

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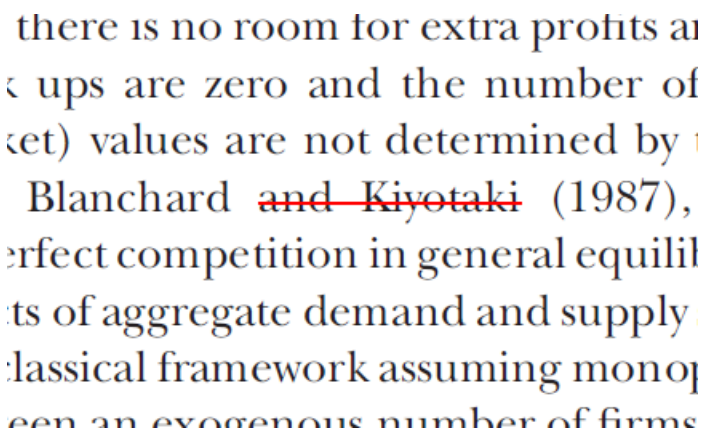

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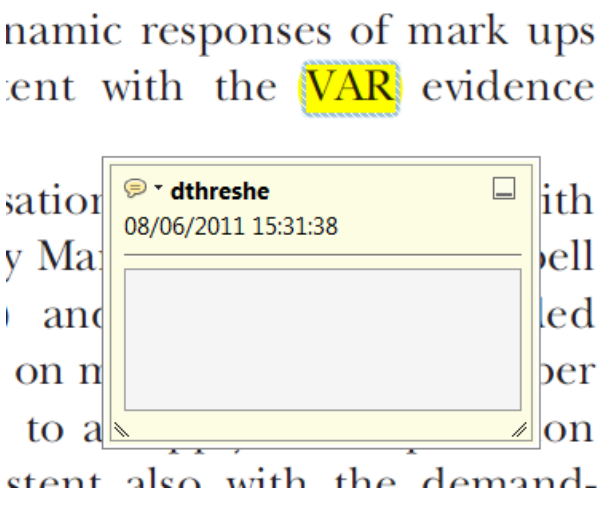

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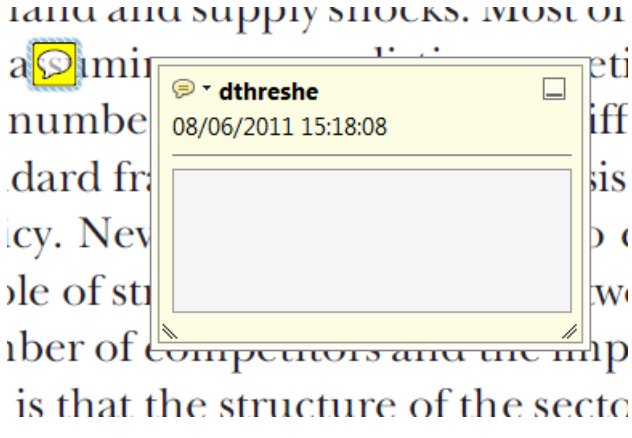

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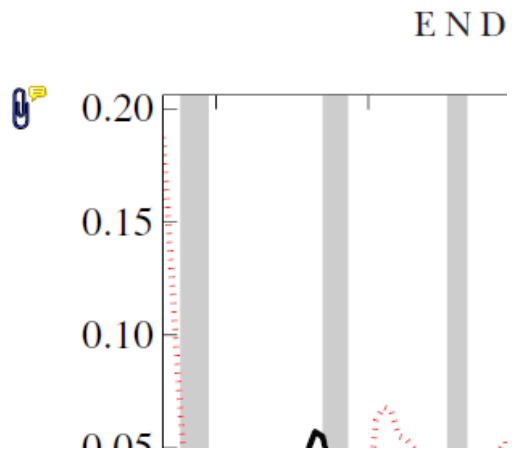

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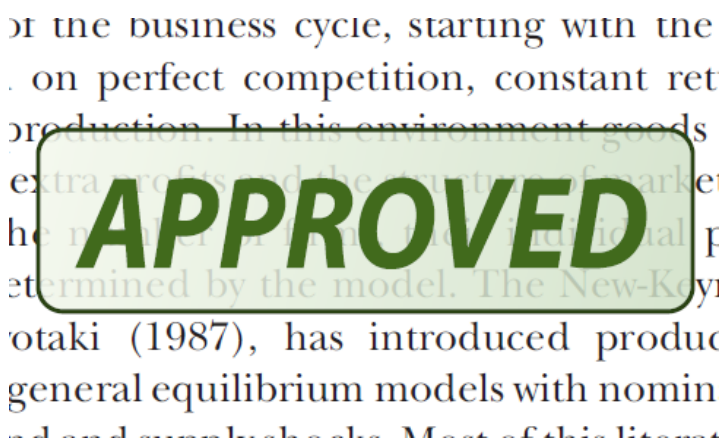

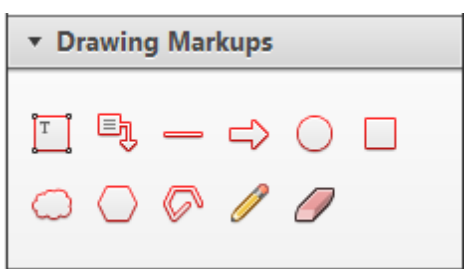

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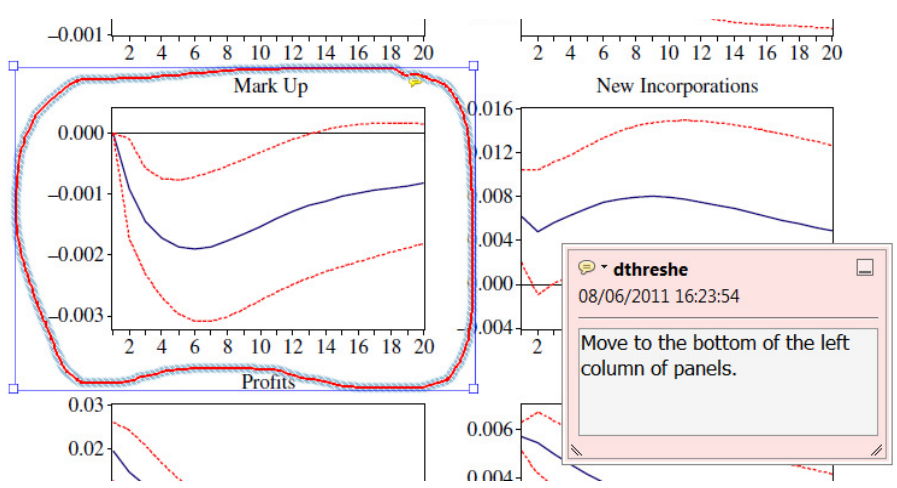

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