Chapter 9

Adenohypophyseal function in bitches treated with medroxyprogesterone acetate

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Abstract

The aim of this study was to investigate the effects of treatment with medroxyprogesterone acetate (MPA) on canine adenohypophyseal function. Five Beagle bitches were treated with MPA (10 mg/kg, every 4 weeks) and their adenohypophyseal function was assessed in a combined adenohypophyseal function test. Four hypophysiotrophic hormones (corticotrophin-releasing hormone, growth hormone (GH)-releasing hormone, gonadotrophin-releasing hormone (GnRH), and thyroid-releasing hormone (TRH)) were administered before and 2, 5, 8, and 11 months after the start of MPA treatment, and blood samples for determination of the plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol, GH, insulin-like growth factor-I (IGF-I), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), α-melanocyte-stimulating hormone (α-MSH), and thyroid-stimulating hormone (TSH) were collected at -15, 0, 5, 10, 20, 30, and 45 min after supra-pituitary stimulation.

Medroxyprogesterone acetate successfully prevented the occurrence of oestrus, ovulation, and a subsequent luteal phase. Treatment with MPA did not affect basal and GnRH-induced plasma LH concentrations. The basal plasma FSH concentration was significantly higher at 2 months after the start of MPA treatment than before or at 5, 8, and 11 months after the start of treatment. The maximal FSH increment and the area under the curve (AUC) for FSH after supra-pituitary stimulation were significantly higher before treatment than at 5, 8, and 11 months of MPA treatment. Differences in mean basal plasma GH concentrations before and during treatment were not significant, but MPA treatment resulted in significantly elevated basal plasma IGF-I concentrations at 8 and 11 months. Treatment with MPA did not affect basal and stimulated plasma ACTH concentrations, with the exception of a decreased AUC for ACTH at 11 months. In contrast, the maximal cortisol increment and the AUC for cortisol after supra-pituitary stimulation were significantly lower during MPA treatment than prior to treatment. Treatment with MPA did not affect basal plasma concentrations of prolactin, TSH, and α-MSH, with the exception of slightly increased basal plasma TSH concentrations at 8 months of treatment. Treatment with MPA did not affect TRH-induced plasma concentrations of prolactin and TSH.

In conclusion, the effects of chronic MPA treatment on adenohypophyseal function included increased FSH secretion, unaffected LH secretion, activation of the mammary GH-induced IGF-I secretion, slightly activated TSH secretion, suppression of the hypothalamic-pituitary-adrenocortical axis, and unaffected secretion of PRL and α-MSH.

Introduction

Progestins, such as medroxyprogesterone acetate (MPA), are commonly used to prevent oestrus in the bitch (Schaefers-Okkens, 1996; Romagnoli and Concannon, 2003). Whether the oestrus-preventing properties of progestins in the bitch are due to effects on the hypothalamus, on the pituitary gland, or at the ovarian level is not clear. McCann et al. (1987) and Colon et al. (1993) reported that basal plasma levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) do not change during progestin treatment. Information about the effect of gonadotrophin-releasing hormone (GnRH) on the secretion of LH during progestin treatment is conflicting. GnRH-induced increases in plasma LH concentrations in progestin-treated dogs did not differ from those in control dogs in one study (Colon et al., 1993), while in another study the GnRH-induced LH levels were reduced (McCann et al., 1987). In dogs there is no information on the effect of progestins on GnRH-stimulated FSH concentrations.

In women, progestins are known to prevent ovulation by inhibiting the mid-cycle surges of FSH and LH, whereas the tonic release of these gonadotrophins continues at luteal phase levels (Mishell, 1996; Jain et al., 2004). Long-term use of depot MPA in women does not affect the pituitary responsiveness of LH and FSH to GnRH administration, suggesting that the pituitary is not the primary site for ovulation inhibition in women (Ismael et al., 1987).

Prolonged treatment with progestins in bitches is associated with alterations in the release of pituitary hormones other than gonadotrophins. Progestin administration leads to a decrease in the pituitary responsiveness of growth hormone (GH) to growth hormone releasing hormone (GHRH) (Watson et al., 1987; Selman et al., 1991, 1994a). This change is due to GH release from foci of hyperplastic ductular mammary epithelium (Selman et al., 1994b; van Garderen et al., 1997), leading to elevated plasma GH levels that do not have a pulsatile plasma profile (Watson et al., 1987). The hypothalamic-pituitary-adrenocortical (HPA) axis is suppressed by progestins (McCann et al., 1987; Rutteman et al., 1987; Selman et al., 1997), due to the intrinsic glucocorticoid properties of progestins (Guthrie and John, 1980; Selman et al., 1996; Selman et al., 1997). While basal plasma concentrations of adrenocorticotrophic hormone (ACTH) are only moderately affected (Selman et al., 1997), the basal plasma concentrations of cortisol are markedly decreased (Concannon et al, 1980; McCann et al., 1987; Rutteman et al., 1989; Selman et al., 1997). In addition, the response of ACTH and cortisol to stimulation with corticotrophin-releasing hormone (CRH) may be

reduced (McCann et al., 1987; Selman et al., 1997). Also, in women the administration of MPA causes suppression of the HPA axis (Jones et al., 1974).

With regard to other anterior pituitary hormones, such as prolactin (PRL) and thyroid-stimulating hormone (TSH), there is little information about the effect of progestin treatment on their release. In the bitch, progestin treatment does not seem to affect mean PRL (Concannon et al., 1980) and TSH concentrations (Frank et al., 1979). Information with regard to pituitary responsiveness of PRL to supra-pituitary stimulation is limited to one study, in which MPA administration did not change PRL response to thyroid-releasing hormone (TRH) in ovariohysterectomized, oestradiol-primed bitches (Rutteman et al., 1987). On the other hand, there is evidence that treatment with MPA increases the pituitary PRL responsiveness to TRH in women (Mishell et al., 1977). Finally, there are no reports on the effect of progestins on the pituitary release of α-melanocyte-stimulating hormone (α-MSH).

The aim of the present study was to obtain an integral picture of the effect of progestins on the function of the adenohypophysis in the bitch. For this purpose, the effects of supra-pituitary stimulation on the release of seven adenohypophyseal hormones were studied before and several times during MPA treatment in Beagle bitches by means of a combined anterior pituitary function test (Meij et al., 1996a),

Materials and methods

Dogs

Studies were carried out in five healthy intact Beagle bitches, aged 3 to 9 years and weighing 9.0 to 10.3 kg, that never had been treated with progestins. They were housed with outdoor access, fed a commercial diet once daily, and given water *ad libitum*. They were accustomed to the laboratory environment and procedures such as collection of blood samples. Throughout the study the general condition of the dogs was monitored by physical examination and routine clinical chemistry.

Study design and blood sample collection

In the dogs used in this study, the tip of the right uterine horn and the corresponding ovary had been excised to serve as control tissues in another study. This surgical procedure had been performed 245 ± 42 days (mean ± standard deviation (SD)) before the start of the treatment with MPA. After the surgery, all of the dogs had had one complete oestrous cycle.

Treatment with the synthetic progestin depot preparation MPA (Depo-Promone®, Pharmacia Animal Health, Puurs, Belgium) was begun during anoestrus in a dose of 10 mg/kg body weight subcutaneously at intervals of 4 weeks, for a total of 13 injections. Three days before the start of the treatment with MPA, the mean plasma progesterone concentration was 0.9 ± 0.3 nmol/l (mean ± SD).

Before and at 2, 5, 8, and 11 months after the start of the treatment with MPA, a combined anterior pituitary function test was performed using four releasing hormones (4RH test) according to methods described previously (Meij et al., 1996a; Meij et al., 1996b). Briefly, an intravenous catheter was placed in the cephalic vein of each dog to facilitate rapid sequential injection. Immediately after the collection of the zero blood sample from the jugular vein, four releasing hormones were injected intravenously within 30 sec, in the following order and doses per kg body weight: 1 µg oCRH (Peninsula Laboratories Inc., Belmont, CA, USA), 1 µg hGHRH (hGHRF; Peninsula Laboratories Inc., Belmont, CA, USA), 10 µg GnRH (Fertagyl®; Intervet, Boxmeer, The Netherlands), and 10 µg TRH (Hoffman-La Roche, Basel, Switzerland). During progestin treatment, the 4RH tests were always performed immediately before the next 4-weekly administration of MPA. The clock for blood sampling was started immediately after the administration of the last releasing hormone. Blood samples were collected at -15, 0, 5, 10, 20, 30, and 45 min from the jugular vein and transferred to ice-chilled EDTA-coated and heparinized (for TSH) tubes. Samples were centrifuged at 4º C for 10 min. Plasma was stored at -25º C until assayed for ACTH, cortisol, GH, FSH, LH, PRL, and TSH. Plasma concentrations of α-MSH and insulin-like growth factor-I (IGF-I) were determined in the -15 and 0 min samples only.

Hormone determination

Plasma progesterone concentrations were measured in a previously validated radioimmunoassay (RIA) (Henry et al., 1987). The sensitivity of the assay was 0.0005 ng. The intra-assay and interassay coefficients of variation were 7.05 % and 8.75 %, respectively.

Plasma ACTH concentrations were measured by use of a two-site immunoradiometric assay (IRMA) (Nichols Institute, Wijchen, The Netherlands). The antiserum is highly specific for $ACTH_{1\text{-}39}$. The intra-assay and interassay coefficients of variation were 3.2 % and 7.8 %, respectively, and the sensitivity of the assay was 0.22 pmol/l. The antiserum cross-reacts with neither α-MSH nor ACTH precursors (Hodgkinson et al., 1984; Findling et al., 1990).

Plasma cortisol concentrations were measured by a RIA validated for the dog (Coat-A-Count® Cortisol, Diagnostic Product Corporation, Los Angeles, CA, USA). Intra-assay and

interassay coefficients of variation ranged from 3.0 to 5.1 % and from 4.0 to 6.4 %, respectively. The sensitivity of the assay was 1 nmol/l.

Plasma FSH concentrations were measured by a homologous canine IRMA (AHC004, Biocode SA, Liège, Belgium). The intra-assay and interassay coefficients of variation for values above 1.6 µg/l were 3.2 % and 15 %, respectively. The sensitivity of the assay was 1.5 µg/l.

Plasma GH concentrations were measured by a commercially available RIA for porcine and canine GH (PGH-46HK; Linco Research, St. Charles MS). The intra-assay coefficient of variation was 7.6 % at a plasma concentration of 4.4 µg/l. The sensitivity of the assay was 1 µg/l

Total plasma IGF-I concentrations were measured in a heterologous RIA, validated for the dog, after acid-ethanol extraction to remove interfering IGF binding proteins (IGFBPs). Plasma IGF was extracted using a mixture of 87.5 % (v/v) ethanol and 12.5 % 2 M formic acid. Tubes containing 100 µl plasma and 400 µl of the ethanol-formic acid mixture were mixed thoroughly and incubated for 30 min at room temperature. After centrifugation for 30 min at 5500 g at 4° C, a 50 µl aliquot of the supernatant was diluted 1:50 with assay buffer containing 63 mM Na_2HPO_4 (pH 7·4), 13 mM Na_2EDTA, and 0·25 % (w/v) BSA. The extraction efficiency was 92.5 ± 5.7 %. Plasma IGF-I concentrations were measured in a heterologous RIA validated for the dog (Favier et al., 2001). The intra-assay coefficient of variation was 8.6 % at a plasma concentration of 100 µg/l. The sensitivity of the assay was 10 µg/l. IGF-I antiserum AFP4892898 and human IGF-I for iodination were obtained from the National Hormone and Peptide Programme (Harbor-UCLA Medical Center, Torrance CA).

Plasma LH concentrations were measured in a heterologous RIA described previously by Nett et al. (1975), with a few modifications. A rabbit antiserum raised against ovine LH (CSU-204, kindly supplied by G D Niswender, Colorado State University, CO, USA), radio-iodinated bovine LH-7981 as prepared for our bovine LH assay (Dieleman and Bevers, 1987), and canine pituitary standard LER 1685-1 (a gift of Dr L E Reichert, Albany Medical College, NY, USA) were used in this assay. The intra-assay and interassay coefficients of variation for values above 0.5 µg/l were 2.3 % and 10.5 % respectively. The sensitivity of the assay was 0.3 µg/l.

Plasma concentrations of α-MSH were measured by RIA without extraction according to methods described previously (Mol et al., 1987). The intra-assay and interassay coefficients of variation were less than 8 % and 23 %, respectively. The sensitivity of the assay was 5 pmol/l.

Plasma PRL concentrations were measured by a previously validated heterologous RIA (Okkens et al., 1985). The intra-assay and interassay coefficients of variation were 3.5 % and 11.5 %, respectively. The sensitivity of the assay was 0.8 µg/l.

Plasma TSH concentrations were measured in a homologous solid-phase, two-site chemoluminescent enzyme immunometric assay (Immulite canine TSH, Diagnostic Products Corporation, Los Angeles, CA, USA) according to the manufacturer's instructions. The intra-assay coefficients of variation were 5.0 %, 4.0 % and 3.8 % at TSH levels of 0.20, 0.50, and 2.6 µg/l, respectively. The interassay coefficients of variation were 6.3 % and 8.2 % at TSH levels of 0.16 and 2.8 µg/l, respectively. The sensitivity of the assay was 0.03 µg/l.

Statistical analysis

Differences in body weight before and after the study were assessed in a paired Student's t-test. Plasma α-MSH concentrations below the limit of quantitation were assigned a value of 5 pmol/l. The following response variables were considered: basal hormone value, maximal increment from basal level, and area under the curve above the basal hormone level (AUC). The basal concentration was defined as the mean of the hormone concentrations at -15 and 0 min. The AUC for hormone concentration following stimulation was calculated by the trapezoidal method. Basal hormone concentration, maximal increment, and AUC before and after MPA treatment were compared for the different hormone concentrations using a mixed model with dog as random effect and period (0, 2, 5, 8, and 11 months after MPA treatment) as categorical fixed effects factor. The periods were compared in pairs, applying Tukey's multiple comparisons technique at a global significance level of 5% to obtain adjusted P values.

Additionally, a mixed model was used with dog as random effect and period, time (min after stimulation) and the period by time interaction as categorical fixed effects to investigate whether the time evolution after stimulation differed from period to period.

Analyses were performed with SAS version 9.1 for Windows (Insightful Corp., Seattle, US).

Ethics of the study

The study protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium.

Results

No signs of oestrus were detected during the 12 months of MPA treatment. In addition, plasma progesterone concentrations were low at 5 months (0.6 ± 0.6 nmol/l; mean ± SD) and 12 months (0.6 ± 0.3 nmol/l) after the start of the treatment. The mean body weight of the dogs on the day of the last MPA injection (12.4 ± 1.6 kg, mean ± SD) was significantly higher (P = 0.02) than that on the day of the first injection of MPA (9.5 ± 0.7 kg).

Basal plasma LH concentrations did not change significantly. In each sampling period, supra-pituitary stimulation resulted in a significant rise (P < 0.001) in plasma LH concentration. The maximal increment and the AUC for LH did not vary significantly with time (Table 1, Figure 1).

Basal plasma FSH concentrations were significantly higher at 2 months after the start of treatment with MPA than before treatment (P = 0.004) or at 5 (P = 0.004), 8 (P = 0.002), or 11 months (P < 0.001) after the start of the treatment. In each sampling period, supra-pituitary stimulation resulted in a significant rise (P < 0.001) in the plasma FSH concentration. The maximal increment was significantly higher before treatment than after 5 (P = 0.01) or 11 months (P = 0.01) of treatment with MPA, while the difference was not significant (P = 0.07) at 8 months. The AUC for FSH was significantly higher before MPA treatment than after 5 (P < 0.001), 8 (P = 0.03), or 11 (P = 0.02) months of treatment with MPA (Table 1, Figure 1).

Table 1. Characteristics of LH and FSH secretion in 5 Beagle dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. The values are expressed as mean ± SEM or median and range. Tmax indicates the time at which maximal supra-pituitary stimulation was observed.

		Months after treatment with MPA			
	Before MPA	2	5	8	11
LH basal (µg/l)	3.0 ± 1.2	4.6 ± 1.9	2.9 ± 0.6	3.9 ± 2.1	2.5 ± 1.1
LH increment (µg/l)	57.4 ± 9.3	58.3 ± 8.9	62.3 ± 10.6	82.9 ± 21.3	82.0 ± 19.3
LH Tmax (min)	10 (5-20)	10 (10-20)	10 (10-20)	10 (10-20)	10 (10-20)
LH AUC (µg/lx45min)	1633 ± 262	1801 ± 363	1797 ± 302	2019 ± 558	2024 ± 386
FSH basal (µg/l)	7.4 ± 0.9	14.2 ± 2.1 **a**	7.5 ± 1.2	6.9 ± 1.2	5.6 ± 0.9
FSH increment (µg/l)	27.2 ± 4.3	22.2 ± 3.8	17.6 ± 3.2 **b**	20.2 ± 4.1	17.5 ± 3.6 **b**
FSH Tmax (min)	20 (10-20)	20 (20-30)	20 (20-45)	20 (10-20)	20 (10-20)
FSH AUC (µg/lx45min)	932 ± 137	756 ± 121	599 ± 116 **b**	645 ± 135 **b**	608 ± 118 **b**

a: significantly different from before treatment and at 5, 8, and 11 months after starting treatment with MPA; **b**: significantly different from before MPA treatment.

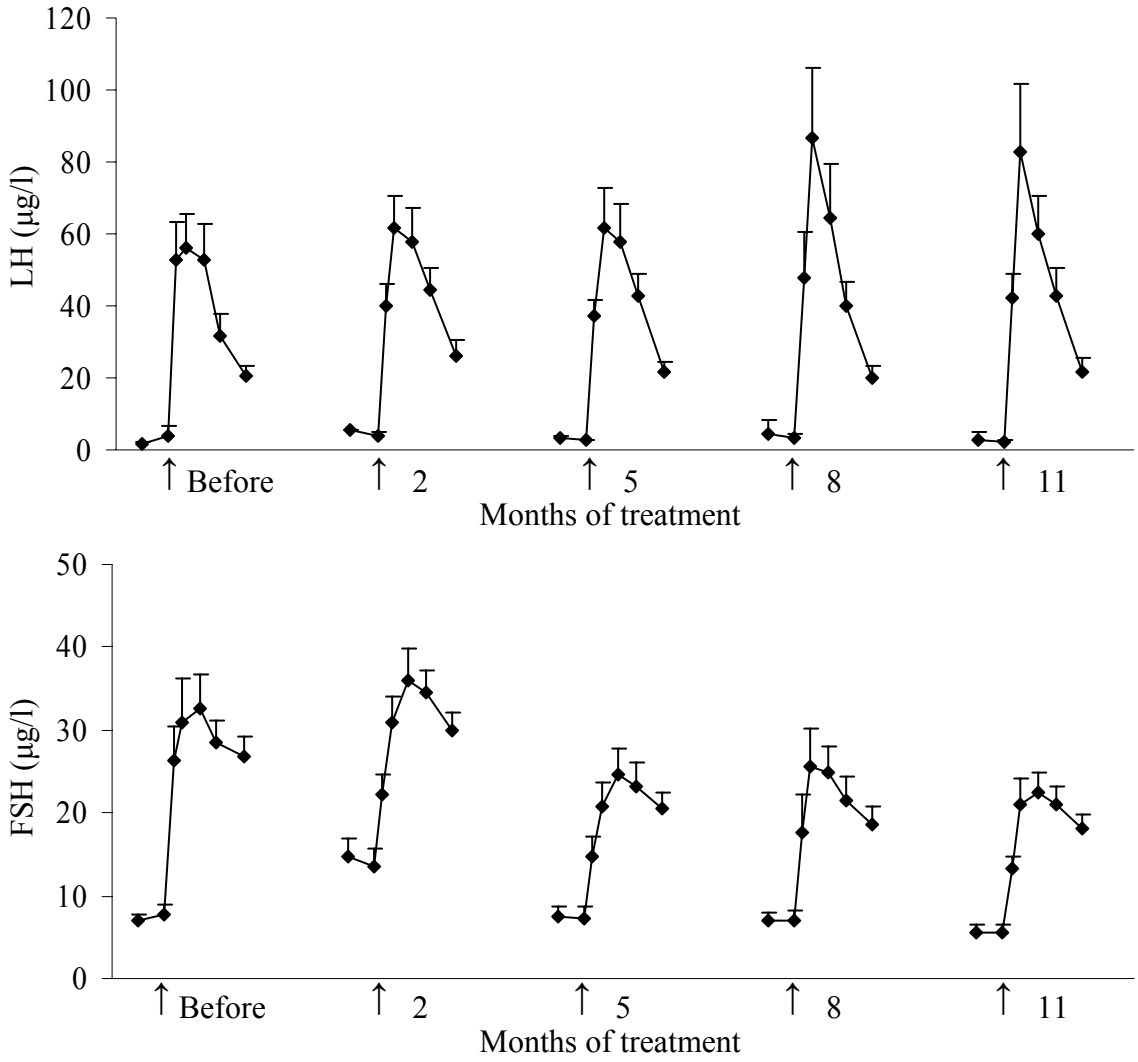

Figure 1. Plasma LH and FSH responses (mean ± SEM) in 5 dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. Blood samples were collected at -15, 0, 5, 10, 20, 30, and 45 min following the injection of the releasing hormones at 0 min (arrow).

Differences in basal plasma GH concentrations before and during treatment with MPA were not significant. At 8 months after the start of treatment with MPA supra-pituitary stimulation resulted in a significant rise ($P < 0.001$) in plasma GH concentration, while no significant effect was noted during the other periods. Differences in the maximal increment and the AUC for GH before and during the treatment with MPA were not significant (Table 2, Figure 2). Basal plasma IGF-I concentrations were significantly higher at 8 months ($P = 0.02$) and 11 months ($P < 0.001$) of treatment with MPA than before treatment. In addition, basal

plasma IGF-I concentratios were significantly higher at 11 months of treatment than at 2 months (P = 0.02) (Table 2).

Table 2. Characteristics of GH and IGF-I secretion in 5 Beagle dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. The values are expressed as mean ± SEM or median and range. Tmax indicates the time at which maximal supra-pituitary stimulation was observed.

		Months after treatment with MPA			
	Before MPA	2	5	8	11
GH basal (µg/l)	2.7 ± 0.9	2.3 ± 0.3	2.5 ± 0.3	2.7 ± 0.4	3.0 ± 0.5
GH increment (µg/l)	1.8 ± 1.5	0.6 ± 0.3	0.9 ± 0.4	1.1 ± 0.2	0.6 ± 0.4
GH Tmax (min)	5 (-15-45)	20 (10-30)	10 (-15-20)	20 (5-45)	10 (0-20)
GH AUC (µg/lx45min)	5.1 ± 44.4	13.1 ± 9.5	23.3 ± 17.6	34.5 ± 7.4	12.4 ± 13.7
IGF-I basal (µg/l)	45 ± 6	108 ± 25	135 ± 35	159 ± 30 **a**	224 ± 53 **a,b**

a: significantly different from before MPA treatment; **b**: significantly different from 2 months after starting treatment with MPA.

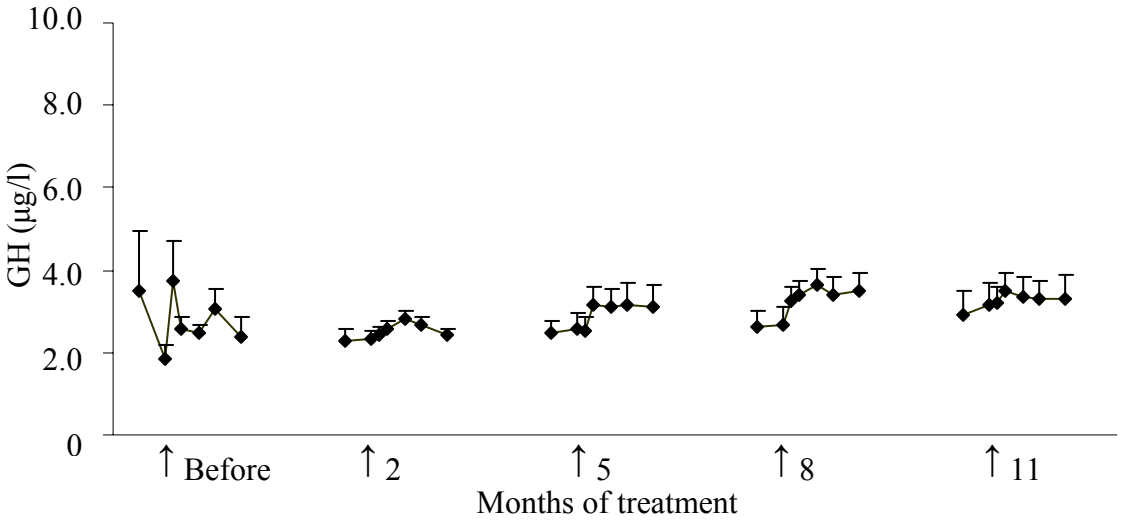

Figure 2. Plasma GH responses (mean ± SEM) in 5 dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. Blood samples were collected at -15, 0, 5, 10, 20, 30, and 45 min following the injection of the releasing hormones at 0 min (arrow).

Differences in basal plasma ACTH concentrations before and during treatment with MPA were not significant. In each sampling period, supra-pituitary stimulation resulted in a significant rise ($P < 0.001$) in plasma ACTH concentration. Differences in the maximal increment before and during the treatment with MPA were not significant. The AUC for ACTH after 11 months of MPA treatment was significantly lower ($P = 0.05$) than at 2 and 8 months after the start of the MPA treatment (Table 3, Figure 3). Differences in basal plasma cortisol concentrations before and during treatment with MPA were not significant. In each sampling period, supra-pituitary stimulation resulted in a significant rise ($P < 0.001$) in plasma cortisol concentration. The maximal increments decreased significantly during treatment ($P = 0.003$, $P = 0.01$, $P = 0.002$, and $P = 0.002$, respectively). The AUC for cortisol in the four periods of treatment with MPA was significantly lower than that before MPA treatment ($P = 0.002$, $P = 0.003$, $P = 0.002$, and $P = 0.002$, respectively) (Table 3, Figure 3).

Table 3. Characteristics of ACTH and cortisol secretion in 5 Beagle dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. The values are expressed as mean ± SEM or median and range. Tmax indicates the time at which maximal supra-pituitary stimulation was observed.

		Months after treatment with MPA			
	Before MPA	2	5	8	11
ACTH basal (pmol/l)	4.0 ± 0.9	6.0 ± 0.7	4.0 ± 0.6	5.0 ± 0.4	5.0 ± 0.4
ACTH increment (pmol/l)	68 ± 14	59 ± 7	47 ± 5	64 ± 8	42 ± 7
ACTH Tmax (min)	5 (5-10)	10 (5-30)	10 (5-30)	10 (5-10)	10 (5-45)
ACTH AUC (pmol/lx45min)	1840 ± 286	2005 ± 212	1498 ± 94	2027 ± 180	1276 ± 231 **a**
cortisol basal (nmol/l)	48 ± 5	60 ± 11	58 ± 18	53 ± 10	58 ± 14
cortisol increment (nmol/l)	380 ± 39	238 ± 12 **b**	258 ± 19 **b**	231 ± 24 **b**	226 ± 16 **b**
cortisol Tmax (min)	30 (30-45)	30 (30-45)	45 (30-45)	30 (20-45)	30 (20-30)
cortisol AUC (nmol/lx45min)	12199 ± 1121	7825 ± 331 **b**	8042 ± 572 **b**	7785 ± 707 **b**	7585 ± 433 **b**

a: significantly different from 2 and 8 months after starting treatment with MPA; **b:** significantly different from before MPA treatment.

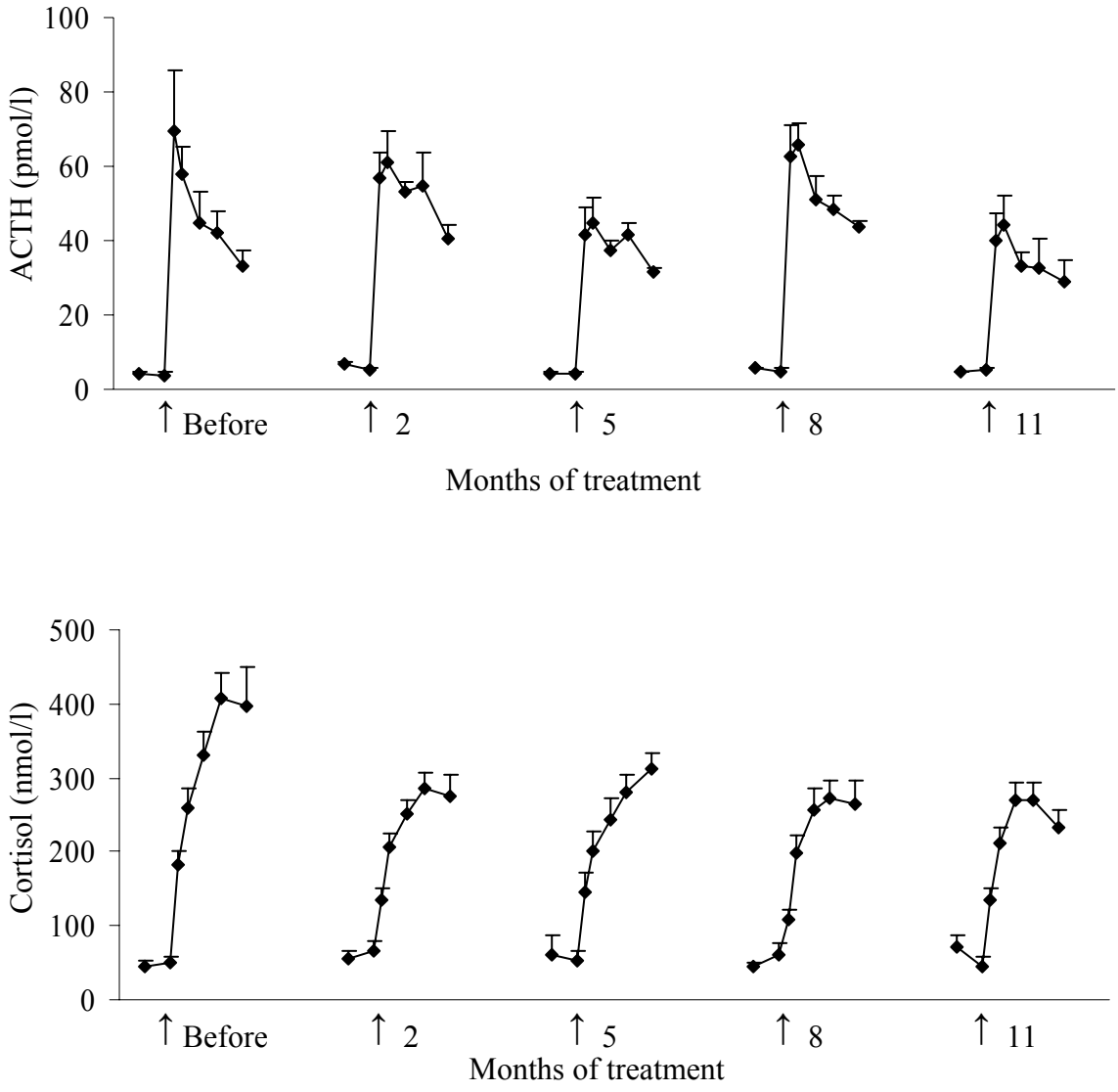

Figure 3. Plasma ACTH and cortisol responses (mean ± SEM) in 5 dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. Blood samples were collected at -15, 0, 5, 10, 20, 30, and 45 min following the injection of the releasing hormones at 0 min (arrow).

Differences in basal plasma PRL concentrations before and during treatment with MPA were not significant. In each sampling period, supra-pituitary stimulation resulted in a significant rise ($P < 0.001$) in plasma PRL concentration. Differences in the maximal increment and the AUC for PRL before and during treatment with MPA were not significant (Table 4, Figure 4).

Basal plasma TSH concentrations at 8 months of MPA treatment were significantly higher than before treatment ($P = 0.03$) and at 5 months of treatment ($P = 0.05$). In each sampling period, supra-pituitary stimulation resulted in a significant rise ($P < 0.001$) in plasma TSH concentration. Differences in the maximal increment and the AUC for TSH before and during MPA treatment were not significant (Table 4, Figure 4).

Basal plasma α-MSH concentrations were 20.7 ± 4.9 pmol/l before treatment and 20.3 ± 5.3, 21.3 ± 5.3, 25.6 ± 8.4, and 32.1 ± 4.4 pmol/l at the four sampling times during MPA treatment. Differences in the mean plasma α-MSH concentrations were not significant.

Table 4. Characteristics of TSH and PRL secretion in 5 Beagle dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. The values are expressed as mean ± SEM or median and range. Tmax indicates the time at which maximal supra-pituitary stimulation was observed.

		Months after treatment with MPA			
	Before MPA	2	5	8	11
TSH basal (µg/l)	0.09 ± 0.03	0.21 ± 0.06	0.14 ± 0.04	0.22 ± 0.08 **a**	0.17 ± 0.07
TSH increment (µg/l)	0.76 ± 0.25	1.01 ± 0.21	0.88 ± 0.17	1.13 ± 0.27	0.91 ± 0.25
TSH Tmax (min)	10 (5-20)	20 (10-30)	30 (10-30)	10 (10-20)	10 (10-30)
TSH AUC (µg/lx45min)	23.4 ± 6.1	34.1 ± 8.0	30.0 ± 5.6	36.2 ± 7.6	28.7 ± 6.1
PRL basal (µg/l)	8.5 ± 2.5	9.1 ± 3.2	11.4 ± 6.7	4.7 ± 0.8	8.0 ± 2.5
PRL increment (µg/l)	53.2 ± 18.0	42.4 ± 14.3	28.5 ± 7.2	66.2 ± 20.1	33.2 ± 6.6
PRL Tmax (min)	10 (5-20)	10 (5-10)	10 (5-20)	10 (5-10)	10 (5-20)
PRL AUC (µg/lx45min)	1086 ± 231	1038 ± 272	698 ± 211	1418 ± 260	806 ± 148

a: significantly different from before MPA treatment and at 5 months of MPA treatment.

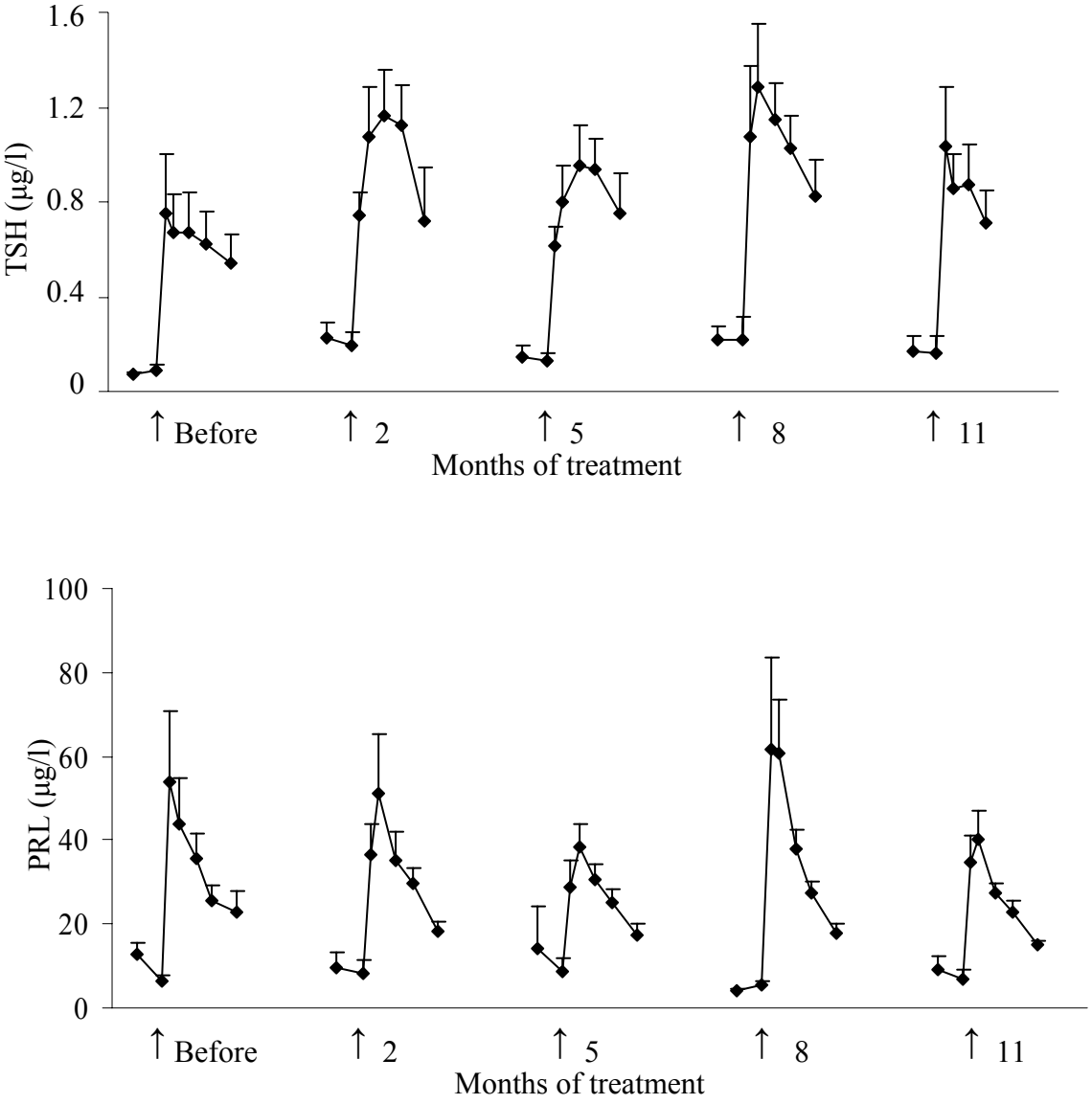

Fig 4. Plasma TSH and PRL responses (mean ± SEM) in 5 dogs in a combined anterior pituitary function test according to Meij et al. (1996a) before and 2, 5, 8, and 11 months after starting treatment with MPA. Blood samples were collected at -15, 0, 5, 10, 20, 30, and 45 min following the injection of the releasing hormones at 0 min (arrow).

Discussion

The tip of one uterine horn and the corresponding ovary were used as control tissues in another study. Nevertheless, all bitches had an oestrous cycle between the surgical procedure and the start of the MPA treatment. Chaffaux and co-workers (1981) demonstrated in dogs

that basal plasma gonadotrophin levels and the response of these hormones to an intramuscular injection of a GnRH analogue were unaffected by unilateral ovariectomy. Furthermore, the difference in plasma FSH concentration between intact and unilateral oophorectomized women was not significant (Cooper and Thorp, 1999). Thus the dogs used in this study can be regarded as having an intact hypothalamus-pituitary-ovarian axis.

The results of this study demonstrate that treatment with MPA affects the hypothalamic-pituitary-ovarian axis. Oestrus, ovulation, and a subsequent luteal phase did not occur in any of the bitches during treatment with MPA, as judged by the lack of external signs of oestrus and low plasma levels of progesterone. The prevention of oestrus by MPA in the present study cannot be ascribed to a significant reduction in circulating levels of either FSH or LH. On the contrary, during the first months of MPA treatment there was an increase in basal plasma FSH without a concomitant change in basal plasma LH. The progestin-induced change in FSH concentration was not observed by Colon et al. (1993) and its recognition may be explained by the repeated sampling employed in the present study.

The results of this study thus indicate that the progestin-induced changes in gonadotrophin release are confined to FSH secretion. One mechanism for the differential control of LH and FSH secretion is gonadal feedback. Both oestradiol and inhibin can specifically suppress FSH synthesis and secretion (Mann et al, 1992; Shupnik, 1996). Reduced secretion of these ovarian hormones can explain the elevated circulating FSH concentration during the first months of MPA treatment. On the other hand, the gonadal peptide activin specifically stimulates FSH secretion. Consequently, a temporary progestin-induced change in the secretion of activin might also explain the initial divergent basal levels of LH and FSH. Other important factors in gonadotrophin control are the frequency and amplitude of GnRH pulses, which have been shown to differentially alter LH and FSH gene expression and secretion (Haisenleder et al., 1991; Shupnik, 1996; Vizcarra et al., 1997).

The elevated basal plasma FSH level during the first months of MPA treatment may thus be due to a direct inhibitory effect of MPA at the ovarian level, resulting in suppression of the ovarian secretion of oestradiol or inhibin, or stimulation of activin release. In this context, increased plasma gonadotrophin levels and low ovarian oestradiol production have been reported in women treated with progestins (Poindexter et al., 1993; Heikinheimo et al., 1996). Observations in monkeys indicate that the inhibitory effects of progesterone on follicular development persist even in the presence of elevated plasma FSH levels, providing additional evidence that progestins may have a direct effect at the ovarian level (Goodman et al., 1982). In women, there are also indications for a hypothalamic site of progestin action

(Couzinet and Schaison, 1993). An initial progestin-induced change in the pattern of hypothalamic secretion of GnRH may therefore be an alternative explanation for the rise in the basal plasma FSH level during the first months of MPA treatment observed in this study.

With continuing MPA treatment, basal plasma FSH returned to pretreatment levels and the pituitary FSH response to supra-pituitary stimulation decreased. These observations may be explained by down-regulation of the pituitary GnRH receptors due to continuous GnRH stimulation (Belchetz et al., 1978). The high GnRH secretion associated with MPA treatment postulated in the previous paragraph may therefore have resulted in desensitization of the response of the gonadotrophs to GnRH. The decline to pretreatment FSH levels and the decrease in responsiveness of pituitary FSH secretion to GnRH are probably part of the oestrus-preventing effects of MPA, because increased FSH secretion is a critical event in the initiation of ovarian folliculogenesis (Kooistra et al., 1999a,b). In other words, MPA treatment for oestrus prevention may prohibit the normal rise in plasma FSH concentration during late anoestrus.

The present results confirm previous findings that progestins alter the GH-IGF-I axis in the bitch (Eigenmann and Rijnberk, 1981). Basal plasma GH concentration tended to increase gradually during the course of the MPA treatment, but the change was not significant. In another study (Concannon et al., 1980), plasma GH concentration did not rise in 27 out of 36 MPA-treated bitches. However, the significant increase in circulating IGF-I concentration during MPA treatment in the present study is consistent with excessive exposure to GH (Selman et al., 1994a). Plasma IGF-I concentration may thus be a more sensitive indicator than plasma GH concentration for the effect of the progestin treatment on the GH-IGF-I axis.

In contrast to previous observations (Meij et al., 1996a), the GH response to supra-pituitary stimulation prior to MPA treatment was not significant. This may be due to ageing (Bhatti et al., 2002), since the dogs in our study were considerably older than those in the study of Meij et al. (1996a). In addition, a relatively high plasma GH level before supra-pituitary stimulation in one of the dogs, probably explained by sampling during a GH pulse, had a substantial influence on basal plasma GH concentration. In agreement with previous observations (Selman et al., 1991), there was no pituitary GH response to supra-pituitary stimulation during MPA treatment in three of the four tests. This may be ascribed to the negative feedback effect of the nonepisodically secreted mammary GH. It has been demonstrated in humans that GH exerts its negative feedback effect by stimulating

hypothalamic somatostatin secretion (Berelowitz et al., 1981). Additionally, the GH-induced elevated circulating IGF-I levels also inhibit pituitary GH secretion (Hartman et al., 1993).

In addition to interacting with the progesterone receptor, MPA also has a relatively high affinity for the glucocorticoid receptor (Selman et al., 1996). Suppression of the HPA axis was thus expected during MPA treatment, as was reported in both humans (Willemse et al., 1990) and dogs (Selman et al., 1994c; Selman et al., 1996). Indeed, we found the cortisol response to stimulation to be decreased with MPA treatment , although ACTH secretion was not demonstrably changed, possible because MPA affects the HPA axis for only 2-3 weeks (Selman et al, 1994c). Although this leads to adrenocortical atrophy (Selman et al, 1997), by 3 weeks (Selman et al., 1994c) or 4 weeks (present study) after administration of MPA, the initially suppressed ACTH (and cortisol) concentrations can have returned to normal.

Treatment with MPA causes significant increases in body weight. The intrinsic glucocorticoid properties of MPA (Selman et al., 1996), leading to increased appetite, may have contributed to this increase in body weight. However, it is difficult to attribute this effect to the glucocorticoid action of MPA alone, since treatment with MPA also affected the GH-IGF-I axis.

As the luteal phase in the bitch progresses, circulating progesterone concentration decreases and PRL secretion increases (Kooistra and Okkens, 2002). This association has also been demonstrated in pregnant and pseudopregnant bitches (Steinetz et al., 1990, Okkens et al. 1997). Moreover, administration of a progesterone receptor antagonist to pregnant bitches causes plasma PRL levels to rise sharply (Galac et al., 2000). Nevertheless, in agreement with previous studies (Concannon et al., 1980; Rutteman et al., 1987), progestin treatment did not affect PRL secretion in the present study. This may be explained by persistently high progestin status.

Mean basal plasma TSH concentration was higher at 8 months after the start of MPA treatment than it was before treatment and at 5 months after the start of treatment. There were no significant alterations in the TSH response to supra-pituitary stimulation. Although basal plasma TSH concentrations were elevated at 8 months after the start of the treatment, they were still within the reference range for TSH in our laboratory. Others found that MPA treatment had no effect on mean circulating TSH levels (Frank et al., 1979), but several authors have reported that total T4 and/or T3 values increase during the progesterone-dominated luteal phase and pregnancy in bitches (Reimers et al., 1984; Dixon, 2004; Feldman and Nelson, 2004). It remains to be seen whether these changes have clinical significance, but

the results of the present study emphasize the importance of considering progestin use when studying the hypothalamic-pituitary-thyroid axis.

In conclusion, MPA successfully prevented the occurrence of oestrus, ovulation, and a subsequent luteal phase. The effects of chronic MPA treatment on adenohypophyseal function included increased FSH secretion, unaffected LH secretion, activation of the mammary GH-induced IGF-I secretion, slightly activated TSH secretion, suppression of the HPA axis, and unaffected secretion of PRL and α-MSH

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