Growth hormone, insulin, glucose, cortisol, luteinizing hormone, and diabetes in beagle bitches treated with medroxyprogesterone acetate

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Abstract. Adult beagle bitches (20 to 101 months old) received medroxyprogesterone acetate (MPA; 75 mg/kg, im) or control vehicle at 3 month intervals. Changes in serum concentrations of GH, insulin and glucose were determined in 18 MPA-treated and 6 of 12 control bitches at 0, 2, 4, 8, 16 and 17–24 months of treatment (Exp. 1). GH, LH and cortisol responsiveness to combined im injection of TRH (10 µg/kg), GnRH (10 µg/kg), and ACTH (5 µg/kg) was determined in 9 MPA-treated and 9 control bitches at 17 months of treatment (Exp. II). In Exp. I, serum concentrations of GH at month 2 (2.6 ± 0.3 µg/l), 4 (3.0 ± 0.3 µg/l), 8 (4.0 ± 1.2 µg/l), 16 (8.5 ± 1.7 µg/l), and 17–24 (21.2 ± 4.1 µg/l) of treatment were greater (P < 0.05) than pretreatment (1.4 ± 0.07 µg/l) and control (1.5 ± 0.1 µg/l) levels. The increase in GH at 2 months preceded (N = 4) or coincided (N = 2) with the development of hyperinsulinaemia and insulin resistance in 6 of the 18 treated bitches, two of which became diabetic by 17 months of MPA treatment. GH (24.6 ± 5.0 vs 11.4 ± 2.1 µg/l) and insulin (308 ± 77 vs 119 ± 9 pmol/l) concentrations were greater (P < 0.05) in older (49 ± 4 months; N = 12) than in the younger (26 ± 2 months; N = 6) treated bitches at 17–24 months of MPA treatment. In Exp. II, pretreatment concentrations of GH were increased (9.8 ± 3.0 vs 1.4 ± 0.1 µg/l, P < 0.01), cortisol levels decreased (12 ± 5 vs 72 ± 10 nmol/l, P < 0.01) and LH concentrations (0.9 ± 0.2 µg/l) unaffected in MPA-treated bitches. After injection of tropic hormones, GH was unchanged, whereas the rises in LH and cortisol levels were less (P < 0.01) in MPA-treated bitches than in control bitches. The results demonstrate that high doses of MPA increase GH concentrations within 2 months and that the GH overproduction and its diabetogenic-like effects were more pronounced in the older than in the younger treated bitches.

Diabetes mellitus and acromegalic symptoms have been reported in dogs chronically treated with gestational compounds (Sloan & Oliver 1975). In women, treatment with medroxyprogesterone acetate (MPA) for periods of 6 to 12 months has resulted in modest, significant increases in concentrations of glucose and insulin (Nash 1975). Diabetes is common in acromegalic humans (Daughaday & Kipnis 1966) and also in normal dogs treated with GH (Altszuler et al. 1968).

Beagle bitches treated with high doses (75 mg/kg every 3 month) of MPA for 17 months had elevated serum growth hormone levels, decreased cortisol levels, acromegalic appearance and a high incidence of mammary gland tumours (Concannon et al. 1980b, 1981). That acromegalic signs were observed in several dogs after 6 months of treatment suggested that GH levels had increased before 17 months of MPA treatment.

This report provides information on the metabolic complications of MPA-induced acromegaly in those and similarly treated dogs. Included are data on the progressive changes in serum concentrations of MPA, GH, glucose and insulin during MPA treatment of intact and ovariectomized...
beagle bitches. The effects of MPA on serum concentrations of LH and on the LH, GH and cortisol responses to combined injection of GnRH, TRH, and ACTH are also reported.

Materials and Methods

Animals

Beagle bitches were 44 ± 3.4 months of age at the start of the study. Bitches were fed a commercial dry ration once daily between 09.00 and 11.00 h and were housed indoors in individual cages. The MPA (Depo-Provera, the Upjohn Co, Kalamazoo, MI) was injected once every 3 months at a dose of 75 mg/kg body weight. Twelve intact and 6 ovariectomized bitches were treated with MPA; 6 of 12 intact control dogs were injected with MPA diluent. Ovariectomies were performed 2 to 24 months before the first injection of MPA. Serum was routinely obtained from blood samples collected by jugular venipuncture between 08.00 and 09.00 h. Blood was allowed to clot in the absence of glycolytic inhibitor for 6 to 8 h at room temperature (20–22°C) before centrifugation.

Necropsies of selected bitches at 17 to 24 months of MPA treatment were performed (Concannon et al. 1981). Bitches were anaesthetized with pentobarbital and exsanguinated before the adrenals and liver were dissected free of extraneous tissue and weighed.

Experiment I. Progressive changes in serum levels of MPA, GH, insulin, glucose and LH.

Concentrations of MPA were determined in intact MPA-treated (N = 12) and control (N = 6) bitches at fortnightly intervals. Serum levels of GH, insulin and glucose were determined at 0, 2, 4, 8, 16–17 (GH only) and 17–24 months in intact (N = 12) and ovariectomized (N = 6) MPA-treated bitches and in diluent-treated control bitches (N = 6). Serum LH levels were determined at 4, 2 and 0 days before and 1, 2, 3, 4, 6, 90, 360 and 450 days after the first injection of MPA in control (N = 12) bitches and MPA-treated intact (N = 12) and ovariectomized (N = 6) bitches.

Experiment II. GH, cortisol and LH responsiveness to tropic stimuli.

Concentrations of GH, cortisol and LH were determined in control (N = 9) and MPA-treated intact (N = 9) bitches at 180, 20 and 1 min before and 5, 10, 20, 40, 80 and 160 min after a combined injection of GnRH (10 µg/kg), ACTH (5 µg/kg), and TRH (10 µg/kg). The combined injection of TRH (Sigma Chemical Co, St. Louis, MO), porcine ACTH (100 IU/mg, grade II; Sigma) and GnRH (Ayerst Research Laboratories, Montreal, Canada) was administered (10.00 h) to overnight fasted dogs at 17 months of treatment, approximately 9 to 10 weeks after the 6th injection of MPA.

Assays

Serum aliquots were stored (−20°C) until assayed for GH, insulin and glucose (Hampshire et al. 1978), LH (Concannon et al. 1980a), cortisol (Concannon et al. 1980b), and progesterone and MPA (Concannon et al. 1981).

Statistical analyses

Differences between groups in GH, insulin and glucose at each sample time in Experiment I were determined by the Wilcoxon-Mann-Whitney test (Steel & Torrie 1960); differences in organ weights among groups were also tested by this method. The univariate correlated t-test for repeated measurements (Gill 1979) determined differences in GH, cortisol and LH among groups in Experiment II. Equality of variance between two treatment groups was done using a two-tailed F test (Steel & Torrie 1960). Student’s paired and unpaired t-test was used where appropriate. Values in the text, figures and tables are means ± SEM except where otherwise indicated.

Table 1.

Mean (± SEM) body weights and weights of liver and adrenals in control and in intact and ovariectomized MPA-treated bitches at 17–24 months of treatment.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (N = 12)</th>
<th>MPA-treated bitches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact (N = 7)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>11.9 ± 0.4</td>
<td>11.6 ± 0.8</td>
</tr>
<tr>
<td>Liver, g</td>
<td>316 ± 12a,b</td>
<td>605 ± 58a</td>
</tr>
<tr>
<td>Adrenals*, g</td>
<td>1.5 ± 0.1a,b</td>
<td>0.94 ± 0.1a</td>
</tr>
</tbody>
</table>

a,b: Means within a row with a common superscript letter differ (P < 0.05).

* Weights of paired adrenals.

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Results

Body weights in control and MPA-treated intact and ovariectomized bitches were similar (Table 1). Liver weights increased ($P < 0.05$) approximately twofold in MPA-treated bitches compared with control bitches (Table 1). In contrast, adrenal weights were reduced ($P < 0.05$) approximately 40% in MPA-treated bitches. These effects of MPA were not affected by prior ovariectomy.

**Experiment 1**

MPA levels in control dogs were not detectable. Serum MPA peaked between 245 and 465 nmol/l during the first 3 to 14 days after injections of MPA and declined exponentially between successive injections (Fig. 1). Mean peak levels of MPA were higher than the peak progesterone levels of 32 to 48 nmol/l found during the 3 to 5 luteal phases exhibited by control bitches during this study (data not shown). During MPA treatment, mean LH levels in treated intact bitches ($0.65 \pm 0.1 \mu g/l$) remained unchanged and were similar ($P > 0.05$) to levels of LH ($0.65 \pm 0.1 \mu g/l$) in control bitches (Fig. 2). The elevated LH levels in ovariectomized bitches ($6.0 \pm 0.2 \mu g/l$) were unchanged during MPA treatment.

Concentrations of GH ($1.5 \pm 0.08 \mu g/l$), insulin ($98 \pm 10 \mu mol/l$), and glucose ($3.6 \pm 0.2 \mu mol/l$) were similar in control and MPA-treated bitches before the first injection of MPA (Fig. 3). Changes in GH, insulin and glucose levels were similar in intact and ovariectomized bitches treated with MPA and so data for all MPA-treated dogs were combined. Levels of GH were greater ($P < 0.05$) in treated than in control bitches throughout MPA treatment (Fig. 3). In MPA-treated bitches, GH increased ($P < 0.05$) from $1.4 \pm 0.07 \mu g/l$ before treatment to $2.6 \pm 0.2 \mu g/l$ at 2 months of treatment and remained elevated at 4 ($3.0 \pm 0.2 \mu g/l$) and 8 ($4.0 \pm 1.2 \mu g/l$) months of treatment before marked increments ($P < 0.01$) occurred at 17 ($8.5 \pm 1.6 \mu g/l$) and 20–24 ($21 \pm 4 \mu g/l$) months of treatment. The magnitude of the GH increment in the MPA-treated bitches appeared related to age. Based on age at start of treatment, levels of GH in the oldest (60 ± 4 months; $N = 6$) bitches and bitches intermediate in age (36 ± 2 months; $N = 6$) were similar but greater than levels in the youngest (26 ± 2 months; $N = 6$) bitches at 17 months ($12.1 \pm 2.5$ vs $7.1 \pm 2.9 \mu g/l$; $P < 0.15$) and 20–24 months ($24.6 \pm 5.0$ vs $11.4 \pm 2.1 \mu g/l$; $P < 0.05$) of treatment.

Concentrations of insulin were not significantly
higher in treated than in control bitches during treatment. However, insulin levels in treated bitches at 17–24 months (264 ± 79 pmol/l) differed \( (P < 0.01) \) from those in control bitches (121 ± 16 pmol/l) using the F-test of the ratio of the two variances. Likewise, variation in glucose concentrations in treated (6.2 ± 1.3 mmol/l) and control (4.8 ± 0.4 mmol/l) bitches differed \( (P < 0.01) \) at 17–24 months of treatment. Age also affected insulin concentrations in the MPA-treated bitches. Insulin levels at 17–24 months of treatment in the oldest and intermediate-aged bitches \( (N = 12) \) were similar \( (308 ± 77 \text{ pmol/l}) \), but greater \( (P < 0.05) \) than those in the six youngest bitches \( (119 ± 9 \text{ pmol/l}) \) treated with MPA or in control bitches \( (117 ± 16 \text{ pmol/l}) \). The development of insulin resistance and/or diabetes in MPA-treated bitches (Table 2) was more common among older \( (N = 5) \) than younger \( (N = 1) \) bitches, but no significant age-group effect was obtained.

**Experiment II**

At 17 months of MPA treatment, concentrations of GH \( (9.8 ± 3.0 \text{ vs } 1.4 ± 0.1 \mu g/l) \) were increased \( (P < 0.01) \), cortisol levels decreased \( (12 ± 5 \text{ vs } 72 ± 10 \text{ nmol/l}, P < 0.01) \), and LH concentrations \( (0.9 ± 0.2 \mu g/l) \) unaffected (Fig. 4). After combined injection of GnRH, ACTH and TRH, concentrations of cortisol and LH increased \( (P < 0.05) \) in each group, whereas GH levels were unaffected. Mean increases in cortisol and LH after injection of tropic hormones were less \( (P < 0.01) \) in MPA-treated than in control bitches.

**Discussion**

The present results extend previous findings of acromegaly, mammary tumours and elevated GH at 17 months of MPA treatment (Concannon et al. 1980b, 1981) by showing that levels of GH, and in some instances insulin, were increased as early as 2 months after the first MPA injection. Thus, increased levels of GH preceded or coincided with early signs of acromegaly and palpable mammary tumours in MPA-treated bitches (Concannon et al. 1980b, 1981).

Mean concentrations of insulin and glucose in the treated bitches were not significantly changed early in the course of MPA-treatment, although some individual bitches were hyperinsulinaemic and mildly hyperglycaemic after 2 or 4 months of MPA treatment. These results agree with the findings of Eigenmann & Rijnberk (1981) who
Progressive changes in concentration (mean ± SEM) of GH, insulin and glucose in control (N = 6) and MPA-treated (N = 18) bitches. Values in treated bitches are pooled data for MPA-treated intact (N = 12) and ovarietomized (N = 6) bitches. GH concentrations in the intact MPA-treated bitches at 17 months of treatment were reported previously (Concannon et al. 1980b).

reported minor changes in insulin and glucose concentrations in bitches treated with MPA for 3 to 4 months. As MPA treatment progressed in our study, however, the incidence and severity of the hyperinsulinaemia increased and coincided with either normal or inappropriately raised levels of glucose in the serum of 6 treated bitches. When the study was terminated after 17–24 months of MPA treatment, two treated bitches clearly had diabetic levels of serum glucose, whereas another four treated bitches had serum glucose and insulin concentrations symptomatic of insulin resistance. The six treated bitches developing insulin resistance and (or) diabetes in this study were identified as acromegalic at 17 months of treatment (Concannon et al. 1980b; P.W. Concannon,

<table>
<thead>
<tr>
<th>Month of study</th>
<th>Dog</th>
<th>Insulin pmol/l</th>
<th>Glucose mmol/l</th>
<th>GH µg/l</th>
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<tr>
<td>2</td>
<td>K131b</td>
<td>210</td>
<td>3.9</td>
<td>2.7</td>
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<tr>
<td></td>
<td>K12</td>
<td>227</td>
<td>4.2</td>
<td>2.3</td>
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<td></td>
<td>K24</td>
<td>192</td>
<td>3.8</td>
<td>2.2</td>
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<td>M90c</td>
<td>227</td>
<td>4.8</td>
<td>3.5</td>
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<tr>
<td></td>
<td>Controld</td>
<td>101 ± 66</td>
<td>4.0 ± 0.7</td>
<td>1.6 ± 0.4</td>
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<tr>
<td>4</td>
<td>K202</td>
<td>262</td>
<td>5.2</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>K131</td>
<td>402</td>
<td>4.8</td>
<td>3.7</td>
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<td></td>
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<td>3.7</td>
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<tr>
<td></td>
<td>Control</td>
<td>101 ± 58</td>
<td>3.9 ± 0.7</td>
<td>1.6 ± 0.7</td>
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<td>6.1</td>
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<td>Control</td>
<td>112 ± 59</td>
<td>4.2 ± 0.8</td>
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<td>K24</td>
<td>473</td>
<td>3.8</td>
<td>63.0</td>
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<tr>
<td></td>
<td>M90</td>
<td>700</td>
<td>3.5</td>
<td>35.3</td>
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<tr>
<td></td>
<td>Control</td>
<td>120 ± 58</td>
<td>4.2 ± 0.8</td>
<td>1.5 ± 0.4</td>
</tr>
</tbody>
</table>

a: MPA-treated bitches were selected on the basis of insulin and/or glucose concentrations that were numerically different from the 99% confidence interval range for the respective control values. Control bitches were 48 ± 6 months old at start of treatment, whereas bitches K202, K131, K12, K93, K24 and M90 were 60, 31, 35, 33, 52 and 34 months old, respectively, at start of treatment.

b: MPA-treated intact bitches designated by prefix K.

c: MPA-treated ovarietomized bitches designated by prefix M.

d: Values in control bitches (N = 6) are means ± 99% confidence interval.

e: The blood sample was obtained at approximately 14.5 months of treatment before bitch K202 was euthanized and necropsied.
The MPA-induced increases in GH preceded or paralleled the development of insulin resistance and diabetes in these bitches. Sloan & Oliver (1975) reported that diabetes and acromegaly occurred in two of sixteen beagle bitches treated with pharmacologic doses of another progestin (chloromadinone acetate). Because of the well-established diabetogenic effects of GH (Daughaday & Kipnis 1966; Altszuler et al. 1968), it seems likely that the elevated GH produced the insulin resistance in this study.

The oldest bitches treated with MPA had the greatest incidence of mammary tumours (Concannon et al. 1980b), and the highest GH levels, and were more likely to develop insulin resistance than were the younger bitches treated with MPA. Such observations suggest that age-related factors affect the extent of GH overproduction and tissue responsiveness to the elevated GH in bitches treated with MPA. Interestingly, the spontaneous occurrence of GH excess and concurrent diabetes mellitus were usually found only in elderly (5 to 13 years old) female dogs (Eigenmann 1981). Furthermore, in dogs and mice, doses of GH that were diabetogenic in adults were ineffective in immature animals (Morgan et al. 1975; Cameron & Kostyo 1987).

The increased liver weight in MPA-treated bitches agrees with the progesterone-induced hepatomegaly observed in several species (Song & Kappas 1968). Elevated levels of GH appear unnecessary for the development of hepatomegaly because increased liver weights (688 ± 58 g) were also found in 6 hypophysectomized bitches similarly treated with MPA (unpublished data). Increased liver mass may have affected hepatic extraction of insulin which is high in the normal dog (Ishida et al. 1984).

The mechanism(s) whereby MPA induces anovulation in the bitch appear(s) to be poorly understood.
related to changes in LH metabolism since basal levels of LH in intact dogs and high levels of LH in ovariectomized dogs were unaffected throughout the 17 months of MPA treatment. The slightly reduced GnRH-induced rise in LH is in agreement with findings showing that 5 of 10 acromegalic patients had a subnormal LH response to GnRH (Cantalamessa et al. 1976). A severe reduction in adrenal size and function within 2 years of MPA treatment was noted sooner than previously reported in dogs treated with MPA for 45 months (Frank et al. 1979). The failure of TRH to increase GH in acromegalic dogs contrasts with the increased GH observed in the majority of acromegalic humans injected with TRH (Müller et al. 1984).

The increased GH in this study could be the result of a direct effect of MPA on the pituitary because gestational compounds increased the size and number of GH-secreting cells in the canine pituitary (Sloan & Oliver 1975; Gräf & El Etreyb 1979). Furthermore, binding of MPA to cytosolic glucocorticoid receptors resulted in increased GH production by rat pituitary tumour cells in vitro (Winneker & Parsons 1981). The marked, stimulatory effects of MPA on GH secretion in bitches contrast with work in humans which showed that progesterone treatment for 5 days (Bhatia et al. 1972) and MPA treatment for 3 or 12 months (Spellacy et al. 1972; Gershberg et al. 1969) resulted in suppression, mild stimulation or no effect, respectively, on GH secretion. Whether age is a confounding influence on tissue responsiveness to MPA in primates is not clear but should be considered based on the greater incidences of acromegaly, mammary tumours, enhanced GH overproduction, and insulin resistance in older than in younger bitches treated with MPA.

Acknowledgments

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References


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