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Effects of the dopamine agonist cabergoline on the pulsatile and TRH-induced secretion of prolactin, LH, and testosterone in male beagle dogs

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Abstract

In the present study, the pulsatile serum profiles of prolactin, LH and testosterone were investigated in eight clinically healthy fertile male beagles of one to six years of age. Serum hormone concentrations were determined in blood samples collected at 15 min intervals over a period of 6 h before (control) and six days before the end of a four weeks treatment with the dopamine agonist cabergoline (5 $\mu g\ kg^{-1}$ bodyweight/day). In addition, the effect of cabergoline administration was investigated on thyrotropin-releasing hormone (TRH)-induced changes in the serum concentrations of these hormones.

In all eight dogs, the serum prolactin concentrations (mean 3.0 ± 0.3 ng ml^{-1}) were on a relatively constant level not showing any pulsatility, while the secretion patterns of LH and testosterone were characterised by several hormone pulses. Cabergoline administration caused a minor but significant reduction of the mean prolactin concentration (2.9 ± 0.2 ng ml^{-1}, $p < 0.05$) and did not affect the secretion of LH (mean 4.6 ± 1.3 ng ml^{-1} versus 4.4 ± 1.7 ng ml^{-1}) or testosterone (2.5 ± 0.9 ng ml^{-1} versus 2.4 ± 1.2 ng ml^{-1}). Under control conditions, a significant prolactin release was induced by intravenous TRH administration (before TRH: 3.8 ± 0.9 ng ml^{-1},

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20 min after TRH: 9.1 ± 5.9 ng ml^{-1}) demonstrating the role of TRH as potent prolactin releasing factor. This prolactin increase was almost completely suppressed under cabergoline medication (before TRH: 3.0 ± 0.2 ng ml^{-1}, 20 min after TRH: 3.3 ± 0.5 ng ml^{-1}). The concentrations of LH and testosterone were not affected by TRH administration.

The results of these studies suggest that dopamine agonists mainly affect suprabasal secretion of prolactin in the dog.

Keywords: Male dog; Prolactin; Basal secretion; TRH-stimulation

1. Introduction

In humans and rats, pituitary prolactin release is both pulsatile and nyctohemeral [1–4]. Also, in female dogs, a pulsatile secretion pattern for prolactin has been demonstrated [5], while in male dogs a pulsatile secretion of this hormone is only suggested considering spontaneously elevated prolactin concentrations found in individual but not all dogs [6]. The pulsatile secretion of pituitary anterior lobe hormones is governed jointly by hypothalamic inhibitory and stimulatory signals [7]. Dopamine has been recognised as the main inhibitory neural signal in the regulation of prolactin release [8,9]. The ergot-alkaloid cabergoline is a potent dopamine-2 receptor agonist. Its suppressing effect on prolactin secretion has been clearly demonstrated in the bitch [10].

In addition to the major inhibitory dopaminergic tone, several substances are known to have prolactin-releasing activity. Only a few years ago, a specific prolactin-release promoting peptide has been identified and characterised in the hypothalamus [11,12]. In addition, several other prolactin-stimulating factors have been reported, such as serotonin [13] and thyrotrophin-releasing hormone (TRH) [14–18]. In a recent study the strong prolactin stimulating effect of TRH was also demonstrated in cattle in vitro and in vivo when compared with the prolactin release induced by a bovine posterior pituitary extract and prolactin releasing peptide [19]. Nevertheless, the exact role of TRH in the physiological regulation of prolactin secretion and the effect of the dopaminergic tone on TRH-induced prolactin secretion are not completely understood [7].

Several studies indicate an interrelationship of the secretion patterns of prolactin and the gonadotrophins. It has been clearly demonstrated that high concentrations of prolactin inhibit GnRH pulsatility in women [20,21] and are associated with decreased gonadotrophin secretion in sows [22]. In females, lowering of the plasma concentration of prolactin to basal level is usually associated with the return of gonadotrophic pulsatility [20,22]. In bitches, administration of dopamine agonists during both the luteal phase and anoestrus results in shortening of the interoestrous interval in the bitch [23–26]. It has been shown that this dopamine agonist-induced shortening of the interoestrous interval in the bitch is associated with an increase in circulating FSH concentration [27].

Also, in men and male rats excessive release of prolactin leads to suppression of gonadotrophin release, which appears to be due to the action of prolactin on the central

nervous system [28]. It has been suggested that prolactin is one of the factors, which regulate the sensitivity of gonadotrophin release to negative testosterone feedback. In hyperprolactinemic men, both LH and testosterone concentrations are reduced, implying increased sensitivity of LH release to negative testosterone feedback [29]. In boars, experimentally induced hyperprolactinemia has been shown to decrease basal LH concentrations without affecting LH pulsatility. The LH decrease was accompanied by an increase in testosterone concentrations [30]. Reduction of serum prolactin concentrations by bromocryptine did, however, not initiate a re-increase in LH concentrations. In contrast, maximum prolactin suppression by bromocryptine in male mice and rats was accompanied by a significant elevation of plasma levels of LH and FSH [31]. It is suggested that a direct stimulatory effect of bromocriptine on testicular steroidogenesis may contribute to its therapeutic effects in hyperprolactinemic men [32]. Data on interaction of prolactin on one hand and the LH-testosterone axis on the other hand are missing in male dogs.

The objective of the present study was to characterise the effects of the dopamine-2 receptor agonist cabergoline on the serum profiles of prolactin, LH, and testosterone in male beagles. In addition, the effects of a single intravenous TRH-injection on the secretion of the three hormones as well as on thyroid-stimulating hormone (TSH) and thyroxine (T_4) were studied before and under cabergoline medication.

2. Materials and methods

2.1. Experimental design

Eight healthy male beagle dogs (A–H) at the age of 12 months to 6 years were included in the study. The dogs were kept in groups of four in roofed over outdoor kennels provided with two shelter huts each. Animal housing, care and experimentation complied with the animal welfare regulations of Germany.

During the three weeks before the start of the study, the dogs were habituated daily to a separate blood collection room, in order to avoid influences on hormone levels induced by stress resulting from environmental factors.

From day 12 until day 41 cabergoline (Galastop®, Vetem S.p.A., Proto Empedocle, Italy) was administered orally once daily at a dosage of 5 $\mu g\ kg^{-1}$. Blood samples for determination of the pulsatile serum profiles of prolactin, LH and testosterone were collected from each dog between 8:30 a.m. and 2:30 p.m. at 15 min intervals at day 1 and day 35. A TRH-stimulation test [33] was performed at day 6 and at day 40. Blood samples were collected immediately before (0 min) and 20, 120, and 180 min after intravenous injection of TRH (Protirelin, TRH-Ferring®, 10 $\mu g\ kg^{-1}$).The blood samples (4 ml each) were collected from the cephalic vein via intravenous catheter. Blood serum was separated by centrifugation and stored at $-20\ ^{\circ}C$ until hormone analyses.

2.2. Hormone analyses

Prolactin concentrations were determined by a validated homologous RIA [23]. Canine prolactin used as standard (B31) was purified from fresh frozen whole pituitary glands [34]

including gel filtration through sephadex G-100. The whole RIA procedure including iodination of porcine prolactin A-7 was essentially the same as reported for porcine prolactin [35]. The minimum detectable concentration of prolactin was 0.8 ng ml^{-1}. The intra- and inter-assay coefficients of variation were 3.5 and 11.8%.

LH concentrations were measured by means of a heterologous RIA using an ovine LH-antibody (GDN no. 15) and a canine LH-standard (LER 1685-1) [36,37]. The sensitivity of the assay was 200 pg ml^{-1} and was defined by the amount of canine LH-standard which significantly prevented binding of the radio-iodinated ovine LH to the LH-antiserum.

Testosterone analysis was performed by RIA in agreement with the method described by [38]. The minimum detectable concentration of testosterone was 20 pg ml^{-1}. The intra- and inter-assay coefficients of variation were 1.1 and 0.5%.

The concentrations of thyroxine and TSH were determined by one-phase chemiluminescence immunoassays (Immulite Canine Total T_4 Assay and Immulite Canine TSH Assay, DPC®, Los Angeles, USA). Laboratory specific reference values for T_4 were 1.3–4.0 μg dl^{-1}, for TSH $\leq$0.5 ng ml^{-1} [33].

2.3. Statistical evaluation

For statistical evaluation, the 6 h serum hormone profiles and the TRH-stimulated hormone concentrations of all eight Beagles were compared before (control) and under cabergoline medication using the paired Student's *t*-test for related samples. Concerning the 6 h serum hormone profiles, the mean concentrations of the 25 control samples and of the 25 samples collected under cabergoline treatment were calculated for each individual dog. Those means ($n = 8$) were then used to derive the means, standard deviations and ranges per group (control and cabergoline). $p < 0.05$ was considered significant.

The 6 h serum profiles of prolactin, LH and testosterone were additionally analysed for pulsatility by means of the Pulsar® programme developed by [39]. The programme identifies hormone peaks by height and duration from a smoothed baseline, using the assay S.D. as a scale factor. The cut-off parameters G1-G5 of the Pulsar® programme were set at 3.98, 2.40, 1.68, 1.24 and 0.93 times the assay S.D. as criteria for accepting peaks 1, 2, 3, 4 and 5 points wide, respectively. The A, B and C values of the Pulsar® programme, used to calculate the assay variance, were set at $A = -0.07$, $B = 3.97$ and $C = 5.66$.

3. Results

3.1. Serum profiles of prolactin, LH and testosterone before and under cabergoline administration

The mean serum prolactin concentration in the first series of blood samples (control) of the eight beagles was 3.0 ± 0.3 (range 2.6–3.5) ng ml^{-1}. Under cabergoline administration, it decreased to 2.9 ± 0.2 (range 2.6–3.2) ng ml^{-1}. The difference was statistically significant ($p < 0.05$).

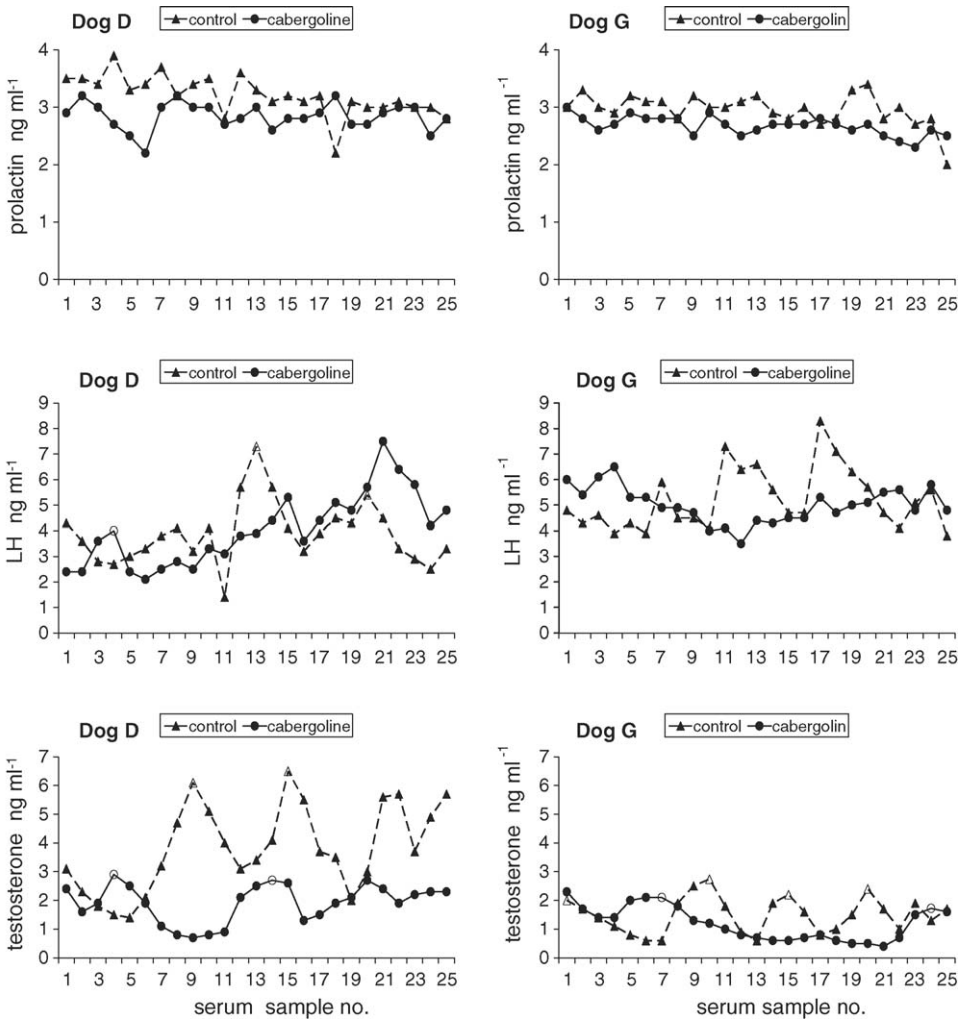

Fig. 1. Six-hour profiles of prolactin, LH and testosterone of two individual male beagles (D, two years; G, six years) without medication (control) and under cabergoline administration. Each series consists of 25 blood serum samples collected at 15 min intervals. LH- and testosterone pulses are indicated by: (Δ) control and (○) cabergoline.

The mean serum concentrations of LH and testosterone were similar before and under cabergoline administration [LH: 4.6 ± 1.3 (range 3.2–7.1) ng ml^{-1} versus 4.4 ± 1.7 (range 2.5–7.7) ng ml^{-1}, testosterone: 2.5 ± 0.9 (range 1.5–3.8) ng ml^{-1} versus 2.4 ± 1.2 (range 0.8–4.2) ng ml^{-1}]. The 6 h secretion patterns of the three hormones before and after cabergoline treatment are illustrated exemplary in two individual dogs (Fig. 1).

Table 1
Number and heights of LH- and testosterone pulses analysed in two series each of 25 blood serum samples collected of eight individual male beagles (A–H) at 15 min interval without medication (control) and under cabergoline administration

Dog	LH				Testosterone			
	Pulses (*n*)		Pulse heights (ng ml^{-1})		Pulses (*n*)		Pulse heights (ng ml^{-1})	
	Control	Cabergoline	Control	Cabergoline	Control	Cabergoline	Control	Cabergoline
A	2	0	5.4, 5.2	–	2	0	4.3, 3.3	–
B	2	0	8.4, 6.7	–	0	0	–	–
C	1	1	14.1	6.1	4	1	1.5, 2.6, 3.3, 3.0	4.5
D	2	1	7.3, 5.4	4.0	2	2	6.1, 6.5	2.9, 2.7
E	0	1	–	5.8	3	1	3.7, 4.2, 3.9	3.9
F	0	1	–	12.2	2	2	3.1, 4.3	2.8, 3.0
G	0	0	–	–	4	2	2.0, 2.7, 2.2, 2.4	2.1, 1.7
H	0	0	–	–	2	3	5.4, 2.5	3.4, 1.0, 1.7
Total	7	4			19	11		

The overall numbers of LH- and testosterone pulses were 7 (control) and 4 (cabergoline) and 19 (control) and 11 (cabergoline), respectively. Hormone pulses were irregularly distributed among animals and experimental periods (Table 1).

3.2. TRH-stimulation test

Intravenous administration of TRH resulted in a significant increase in the serum concentrations of TSH (before TRH: 0.1 ± 0.01 ng ml^{-1}, 20 min after TRH: 0.4 ± 0.1 ng ml^{-1}) and thyroxine (before TRH: 1.5 ± 0.5 μg dl^{-1}, 120 min after TRH: 2.2 ± 0.5 μg dl^{-1}, 180 min after TRH: 2.7 ± 0.7 μg dl^{-1}). Under cabergoline treatment, similar TRH-induced rises in the serum concentrations of TSH and thyroxine were found (TSH: before TRH 0.08 ± 0.02 ng ml^{-1}, 20 min after TRH 0.3 ± 0.1 ng ml^{-1}; thyroxine: before TRH 1.2 ± 0.5 μg dl^{-1}, 120 min after TRH 1.9 ± 0.4 μg dl^{-1}, 180 min after TRH 2.4 ± 0.5 μg dl^{-1}).

Before cabergoline administration, the intravenous injection of TRH induced a marked stimulation of prolactin secretion represented by an increase of the mean blood serum concentration from 3.8 ± 0.9 to 9.1 ± 5.9 ng ml^{-1} 20 min later (Fig. 2). The individual variation of basal and stimulated prolactin values was 2.4–4.9 and 3.0–19.8 ng ml^{-1}, respectively. The serum prolactin concentration subsequently decreased to the initial level within 180 min after TRH injection.

A corresponding TRH-induced prolactin increase did not become evident under cabergoline medication, resulting in a significant difference between the two prolactin values found 20 min after TRH administration ($p < 0.05$, Fig. 2).

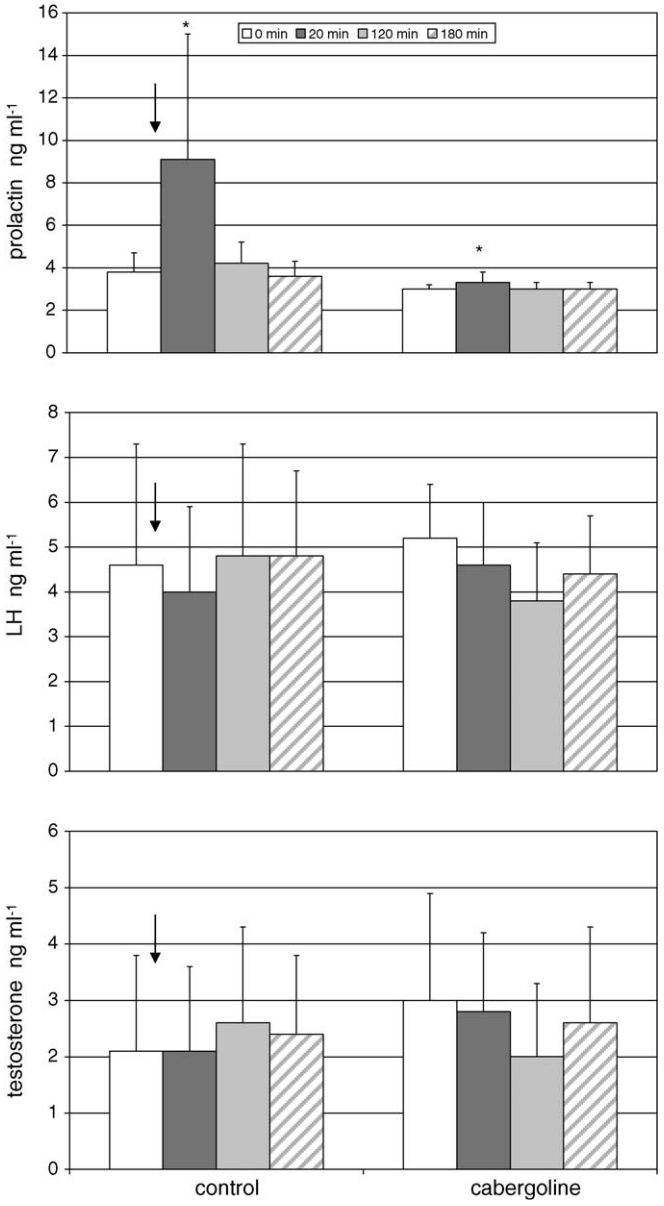

Fig. 2. Mean concentrations (±S.D.) of prolactin, LH and testosterone in blood serum samples of eight healthy mature male beagles before (0 min) and 20, 120 and 180 min after intravenous TRH-injection (arrow) without medication (control) and under cabergoline administration. The TRH induced prolactin increase was significantly different ($^{*}p < 0.05$).

Serum concentrations of LH and testosterone showed a high individual variety both before and after TRH-injection (Fig. 2).

4. Discussion

The mean basal prolactin concentrations measured in the eight male beagles were slightly different from those found in other studies. The mean annual prolactin concentration in 16 male mongrel dogs was 4.6 ± 0.8 ng ml^{-1} [40]. A mean prolactin concentration of 1.7 ± 0.2 ng ml^{-1} has been reported in a group of six cross-bred and two pure-bred dogs using a commercial homologous endpoint EIA [6]. These may be due to breed peculiarities, differences in sampling frequency (number of samples per time period) as well as assay systems.

The reduction of the mean prolactin concentration obtained by a three week cabergoline administration at a dosage of 5 μg kg^{-1}, which is known to be efficient in treating hyperprolactinemia in overt pseudopregnant bitches [41] was, although significant, surprisingly small. This and the complete lack of prolactin pulses may indicate that the prolactin secretion before cabergoline treatment was really basal. In contrast to another study [6], in which occasional distinct elevations in prolactin concentration in individual male dogs of different breeds were reported in a time window of 2.5 h, we identified in beagle dogs just a smoothly oscillating baseline without significant pulses over 6 h. In beagle bitches some prolactin pulses could be identified even in anoestrus, the period of lowest basal prolactin secretion in female dogs [42].

In humans it has been demonstrated that the secretion pattern of prolactin is the result of both basal/non pulsatile (or constitutive) and pulsatile prolactin release, whereby the basal prolactin secretion presumptively mirrors functional pituitary lactotroph cell secretory mass and the pulsatile prolactin release reflects intermittent hypothalamic stimulatory input and episodic withdrawal of brain inhibitory signals to responsive lactotroph cells [4]. Consequently, the secretion pattern of prolactin in our male dogs may just reflect constitutive prolactin release and thus is non-pulsatile.

The mean basal concentrations and pulse frequencies of LH and testosterone measured in 25 blood serum samples collected over a 6 h period corresponded well with the findings previously reported for beagles of the same age. The intra- and interindividual variations observed in dogs from our colony have been reported before [38,43]. In-line with observations in boars, in which bromocryptine-induced hypoprolactinemia did not alter LH secretion patterns [30], the cabergoline-induced prolactin suppression in our dogs had no effect on the secretion patterns of LH. In the bitch it has been demonstrated that shortening of the interoestrous interval by the dopamine agonist bromocryptine is due to a direct stimulating effect of dopamine on GnRH secretion and a subsequent gonadotrophin release [44]. From the results of the present study, a corresponding effect could not be verified. The marked difference of testosterone pulses found under control conditions ($n = 19$) and under cabergoline treatment ($n = 11$) may point to a suppressing effect of cabergoline administration on testosterone pulse frequency. Nevertheless, this seems doubtful, as the contrary is reported both with regard to an indirect (gonadotrophin-mediated) and a direct stimulatory effect of dopamine agonists on testicular

steroidogenesis in male individuals of different species [31,32]. Therefore, it is concluded that in our dogs the cabergoline-induced minor reduction of basal prolactin secretion did not affect testosterone secretion.

The TRH induced increases of TSH and thyroxine indicate normal function of the anterior pituitary-thyroid axis. The sharp rise of prolactin concentrations measured in coincidence with the TSH rise 20 min after intravenous TRH injection shows the likewise stimulation of thyrotrophic and lactotrophic pituitary cells and confirms that TRH is a potent PRF in the dog [33] as described in other species (sheep [14]; rat [17]). Suppression of this TRH-mediated stimulation of prolactin release by cabergoline suggests dopaminergic suppression of the sensitivity of the lactotrophs towards TRH. Accordingly, the sensitivity of prolactin secreting cells towards TRH was found to increase by reduction of dopamine concentrations [7]. The minimal suppression of the basal prolactin secretion and the marked suppression of the TRH-induced hyperprolactinemia during cabergoline administration illustrate that the effects of cabergoline are mainly directed at the pulsatile prolactin release, i.e. cabergoline mainly influences the hypothalamic regulation of prolactin release by the pituitary anterior lobe.

TRH-induced hyperprolactinemia has been shown to last for at least 90 min with slightly decreasing tendency in castrated calves [19], but may have been shorter in the intact dogs, as after 120 min serum prolactin concentrations had almost decreased to basal levels. Whether or not this short prolactin rise is causally related to the slight but not significant decrease of the mean LH concentration 20 min after TRH is questionable and cannot be concluded from the present data.

Abnormally high prolactin concentrations have been found to inhibit gonadotrophin secretion in rats [45,46] and boars [30]. The mechanism of gonadotrophin suppression seems to be located in the hypothalamus, where the tonic GnRH activity is reduced by increased prolactin concentrations leading to an inhibition of the pulsatility of LH secretion [47]. In hyperprolactinemic men, both LH and testosterone levels are reduced implying increased sensitivity of LH to negative testosterone feedback [29]. Dopaminergic treatment in hyperprolactinemic men is suggested to directly stimulate testicular steroidogenesis and by this contribute to the therapeutic effects [32].

The cabergoline-induced suppression of TRH-induced hyperprolactinemia shown in this study and the suppression of hyperprolactinemia in pseudopregnant bitches [41] suggest that suppression of prolactin secretion by cabergoline or another dopamine agonist may also be an effective method to improve fertility in hyperprolactinemic conditions in male dogs. Aetiology and diagnosis of such conditions have, however, not been established in this species. Therefore, further investigations are needed with regard to breed and fertility related prolactin secretion as well as to dopaminergic prolactin suppression in cases of hyperprolactinemia.

In conclusion, the results of the present study demonstrate that administration of the dopamine agonist cabergoline only has a minor suppressing effect on mean serum prolactin concentrations. However, the dopamine agonist cabergoline markedly suppressed TRH-induced suprabasal prolactin secretion. The results of this study did not provide evidence for interrelationships between prolactin and LH/testosterone.

References

[1] Sassin JF, Frantz AG, Weitzman ED, Kapen S. Human prolactin: 24-hour pattern with increased release during sleep. Science 1972;177:1205–7.
[2] Shin SH, Shi HJ. Unsuppressed prolactin secretion in the male rat is pulsatile. Neuroendocrinology 1979;28:73–81.
[3] Lafuente A, Marco J, Esquifinio AI. Pulsatile prolactin secretory patterns throughout the oestrous cycle in the rat. J Endocrinol 1993;137:43–7.
[4] Iranmanesh A, Mulligan T, Veldhuis JD. Mechanisms subserving the physiological nocturnal relative hypoprolactinemia of healthy older men: dual decline in prolactin secretory burst mass and basal release with preservation of pulse duration, frequency, and interpulse interval. A general clinical research study. J Clin Endocrinol Metab 1999;84:1083–90.
[5] Kooistra HS, Okkens AC. Secretion of growth hormone and prolactin during progression of the luteal phase in healthy dogs: a review. Mol Cell Endocrinol 2002;197:167–72.
[6] Corrada Y, Castex G, Sosa Y, Gobello C. Secretory patterns of prolactin in dogs: circannual and ultradian rhythms. Reprod Dom Anim 2003;38:219–23.
[7] Neill JD, Nagy GM. Prolactin secretion and its control. In: Knobil E, Neill J, editors. The physiology of reproduction. New York: Raven Press; 1994. p. 1833–60.
[8] Ben-Jonathan N. Dopamin: a prolactin-inhibiting hormone. Endocrinol Rev 1985;6:564–89.
[9] Lopez FJ, Dominguez JP, Sanchez-Franco F, Negro-Vilar A. Role of dopamine and vasoactive intestinal peptide in the control of pulsatile prolactin secretion. Endocrinology 1989;124:527–35.
[10] Grünau B. Vergleichende Untersuchung zur Behandlung der Pseudogravidität der Hündin mit Prolaktin-hemmern. Diss Med Vet, Tierärztliche Hochschule Hannover 1994.
[11] Hinuma S, Habata Y, Fujii R, Kawamata Y, Hosoya M, Fukusumi S, et al. A prolactin-releasing peptide in the brain. Nature 1998;393:272–6.
[12] Matsumoto H, Noguchi J, Horikoshi Y, Kawamata Y, Kitada C, Hinuma S, et al. Stimulation of prolactin release by prolactin-releasing peptide in rats. Biochem Biophys Res Commun 1999;259:321–4.
[13] Garthwaite TL, Hagen TC. Evidence that serotonin stimulates a prolactin-releasing factor in the rat. Neuroendocrinology 1979;29:215–20.
[14] Thomas GB, Cummins JT, Griffin N, Clarke IJ. Effect and side of action on hypothalamic neuropeptides on prolactin in sheep. Neuroendocrinology 1988;48:252–7.
[15] Lamberts SW, Macleod RM. Regulation of prolactin secretion at the level of the lactotroph. Annu Rev Physiol 1990;70:279–318.
[16] Ben-Jonathan N, Laudon M, Garris PA. Novel aspects of posterior pituitary function: regulation of prolactin secretion. Front Neuroendocrinol 1991;12:231–77.
[17] Lafuente A, Marco J, Esquifinio AI. Physiological roles of thyrotropin-releasing hormone and vasoactive intestinal peptide on the pulsatile secretory patterns of prolactin in pituitary-grafted female rats. J Endocrinol 1994;142:581–6.
[18] Meij BP, Mol JA, Rijnberk A. Thyroid-stimulating hormone response after single administration of thyrotropin-releasing hormone and combined administration of four hypothalamic releasing hormones in beagle dogs. Domest Anim Endocrinol 1996;13:465–8.
[19] Hashizume T, Sasaki T, Nonaka S, Hayashi T, Takisawa M, Horiuchi M, et al. Bovine posterior pituitary extract stimulates prolactin release from the anterior pituitary gland in vitro and in vivo in cattle. Reprod Dom Anim 2005;40:184–9.
[20] Saunder SE, Frager M, Case GD, Kelch RP, Marshall JC. Abnormal patterns of pulsatile luteinizing hormone secretion in women with hyperprolactinemia and amenorrhea: responses to bromocriptine. J Clin Endocrinol Metab 1984;59:941–8.
[21] Yazigi RA, Quintero CH, Salameh WA. Prolactin disorders. Fertil Steril 1997;67:215–25.
[22] Bevers MM, Willemse AH, Kruip TAM. The effect of bromocryptine on luteinizing hormone levels in the lactating sow: evidence for a suppressive action by prolactin and the suckling stimulus. Acta Endocrinol 1983;104:261–5.
[23] Okkens AC, Bevers MM, Dieleman SJ, Willemse AH. Evidence of the non involvement of the uterus in the lifespan of the corpus luteum in the cyclic dog. Vet Quart 1985;7:169–73.

[24] Van Haaften B, Dieleman SJ, Okkens AC, Bevers MM, Willemse AH. Induction of oestrus and ovulation in dogs by treatment with PMSG and/or bromocriptine. J Reprod Fertil 1989;(Suppl. 39):330–1.
[25] Concannon PW. Biology of gonadotrophin secretion in adult and prepupertal female dogs. J Reprod Fertil 1993;(Suppl. 47):3–27.
[26] Onclin K, Verstegen J, Silva LDM, Concannon P. Patterns of circulating prolactin, LH and FSH during dopamine-agonist induced termination of anestrus in beagle dogs. Biol Reprod 1995;52(Suppl. 1) [abstract 314].
[27] Kooistra HS, Okkens AC, Bevers MM, Popp-Snijders C, van Haaften B, Dieleman SJ, et al. Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrus in beagle bitches. Biol Reprod 1999;60:65–71.
[28] Bartke A, Klemcke H, Matt K. Effects of physiological and abnormally elevated prolactin levels on the pituitary-testicular axis. Med Biol 1986;63:264–72.
[29] Bartke A, Matt KS, Steger RW, Clayton RN, Chandrashekar V, Smith MS. Role of prolactin in the regulation of the hypothalamic-pituitary system to steroid feedback. Adv Exp Med Biol 1987;219:153–75.
[30] Jedlinska M, Rozewiecka L, Ziecik AJ. Effect of hypoprolactinaemia on LH-secretion, endocrine function of testis and structure of seminiferous tubules in boars. J Reprod Fertil 1995;103:265–72.
[31] Rao MR, Bartke A, Parkening TA, Collins TJ. Effect of treatment with different doses of bromocriptine on plasma profiles of prolactin, gonadotrophins and testosterone in mature male rats and mice. Int J Androl 1984;7:258–68.
[32] Bartke A, Lackritz RM. Bromocriptine stimulates testosterone production by mouse testes in vitro. Fertil Steril 1981;35:472–6.
[33] Hoppen HO, Lohmann P, Schlote S, Günzel-Apel AR, Müller-König A, Grünau B, et al. Die Messung von caninem TSH zur Diagnostik der Hypothyreose des Hundes. Der praktische Tierarzt 1997;78:13–7.
[34] Reichert LE. Purification of anterior pituitary hormones. In: Colowick SP, Kaplan NO, editors. Methods of enzymology XXXVII. New York: Academic Press; 1975. p. 360–81.
[35] Bevers MM, Willemse AH, Kruip TAM. Plasma prolactin levels in the sow during lactation and the post-weaning period as measured by radioimmunoassay. Biol Reprod 1978;19:628–34.
[36] Nett TM, Akbar AM, Phemister RD, Holst PA, Reichert Jr LE, Niswender GD. Levels of luteinizing hormone, estradiol and progesterone in serum during the estrous cycle and pregnancy in the beagle bitch. Proc Soc Exp Biol Med 1975;148:136–9.
[37] Okkens AC, Bevers MM, Dieleman SJ, Willemse AH. Evidence for prolactin as the main luteotrophic factor in cyclic dogs. Vet Quart 1990;12:193–201.
[38] Günzel-Apel AR, Brinckmann HG, Hoppen HO. Dynamik der LH- und Testosteron-Sekretion bei Beagle-Rüden verschiedener Altersgruppen. Reprod Dom Anim 1990;25:78–86.
[39] Merriam GR, Wachter KW. Algorithms for the study of episodic hormone secretion. Am J Physiol 1992;243:E310–8.
[40] Shafik A. Prolactin injection, a new contraceptive method: experimental study. Contraception 1994;50: 191–9.
[41] Jöchle W, Arbeiter K, Post K, Ballabio R, D'Ver ASD. Effects on pseudopregnancy, pregnancy and interoestrous intervals of pharmacological suppression of prolactin secretion in female dogs and cats. J Reprod Fertil 1989;(Suppl. 39):199–207.
[42] Kooistra HS, Okkens AC, den Hertog E, Mol JA, Dieleman SJ, Rijnberk A. Pulsatile secretion patterns of prolactin and α-melanocyte-stimulating hormone during the luteal phase and mid-anoestrus in beagle bitches. In: Kooistra H, editor. Adenohypophyseal function in healthy dogs and dogs with pituitary disease. Ph.D. Thesis. The Netherlands: University of Utrecht, 2000. p. 135–52.
[43] Günzel-Apel AR, Hille P, Hoppen HO. Spontaneous and GnRH-induced pulsatile LH and testosterone release in pubertal, adult and aging male Beagles. Theriogenology 1994;41:737–45.
[44] Beijerink N, Dieleman SJ, Kooistra HS, Okkens AC. Low doses of bromocriptine shorten the interestrous interval in the bitch without lowering plasma prolactin concentration. Theriogenology 2003;60: 1379–86.
[45] Hafiez AA, Lloyd CW, Bartke A. The role of prolactin in regulation of testis function: the effect of prolactin and luteinizing hormone on the plasma levels of testosterone and androstenedione in hypophysectomized rats. J Endocrinol 1972;52:327–32.

[46] Bartke A, Smith MS, Michael SD, Peron FG, Dalterio S. Effects of experimentally-induced chronic hyperprolactinaemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology 1977; 100:182–6.
[47] Bouchard P, Lagoguey M, Brailly S, Schaison G. Gonadotropin-releasing hormone pulsatile administration restores luteinizing hormone pulsatility and normal testosterone levels in males with hyperprolactinemia. J Clin Endocrinol 1985;60:258–62.