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Testicular gene expression of steroidogenesis-related factors in prepubertal, postpubertal, and aging dogs

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ABSTRACT

Developmental and aging changes in testicular factors related to steroidogenesis are unknown in dogs. Using reverse transcription quantitative real-time PCR, this study examined testicular mRNA levels of *CYP11A1* (P450 cholesterol side-chain cleavage enzyme, P450scc), *CYP17A1* (P450 17α-hydroxylase/C17–20 lyase, P450c17), *HSD3B2* (3β-hydroxysteroid dehydrogenase, 3β-HSD), *CYP19A* (P450 aromatase, P450arom), *STAR* (steroidogenic acute regulatory protein, StAR), cyclooxygenase (COX) -1 and COX-2 in prepubertal (4–6 months of age), postpubertal (1 year of age), and aging (2–18 years of age) dogs. Testicular mRNA levels for P450scc, 3β-HSD, StAR, COX-1, and COX-2 did not change from prepubertal to postpubertal stages, whereas that for P450arom markedly and abruptly increased and that for P450c17 gradually decreased. In postpubertal and aging dogs, a negative correlation was found between aging and testicular P450arom mRNA levels. Based on the rapid testicular growth observed during puberty, these results suggested that total testis gene expression for steroidogenesis-related factors, in particular for P450arom, increases during puberty in dogs. In addition, the decline in P450arom gene expression during aging may affect the ability to synthesize steroids in canine testes.

1. Introduction

Reproductive function changes during sexual maturation and aging in mammalian species. In males, androgen, which is secreted mainly from Leydig cells by stimulation of LH, plays critical roles in these processes and in the maintenance of reproductive functions [1]. Estrogen is also indispensable for male fertility because it has essential roles in spermatogenesis [2,3]. Binding of LH to its specific receptor initiates a cascade of events that includes activation of adenylyl cyclase leading to increased production of cAMP and activation of cAMP-dependent protein kinase [4]. This signal increases the expression and activity of steroidogenic acute regulatory (StAR) protein, which shuttles cholesterol from the outer to the inner mitochondrial membrane, a critical step in the initiation of steroidogenesis [5]. Cholesterol is metabolized by cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) to pregnenolone, which is followed by further metabolization due to enzymes such as cytochrome P450 17α-hydroxylase/C17 to 20 lyase (P450c17), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-HSD, and cytochrome P450 aromatase (P450arom), resulting in the production of androgen and estrogen [6]. In the canine testes, testosterone is produced mainly through the Δ5-steroid pathway [7].

During sexual maturation in dogs, blood concentrations of androgen increase in association with an increase in LH receptor expression in the testis [8], with blood LH concentrations in pubertal dogs higher than those in adult dogs [9]. The estrogen concentration in peripheral blood also increases during sexual maturation in human boys [10,11] and male monkeys [12] and boars [13], whereas it has not been studied in male dogs. During aging, Leydig cell

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function [14–17] and blood testosterone concentration [15,16] decline in rats, and testosterone concentration in peripheral blood decreases in human males [18]. A decreased ability of Leydig cells to produce steroid hormones is correlated with a reduction in the levels and activities of some steroidogenic enzymes [14,15,17] and with reduced levels of mRNAs for StAR in older rats [15–17]. In dogs, although no decline has been detected in peripheral blood testosterone concentration with aging [19,20], a negative correlation between aging and estrogen levels in testicular venous blood has been shown [19]. However, the changes in testicular expression of steroidogenic factors during sexual maturation and aging have not been studied.

Inhibition of cyclooxygenase (COX)-2 activity enhances StAR gene expression and steroidogenesis in mouse Leydig cells [21], and an increase in COX-2 expression during aging is associated with decreased StAR expression and decline of testosterone production in rat testes [16]. Both the two isoforms of COX (i.e., COX-1 and COX-2) catalyze a key step in the conversion of arachidonate to prostaglandin (PG) H_2, the immediate substrate for a series of cell-specific PGs; intratesticular injections with $PGF_{2\alpha}$ inhibit testosterone production in rat testes [22]. These findings suggest that PG generated via COX inhibits steroid production through decreased expression of StAR in the testis. However, testicular expression of StAR, COX-1, and COX-2 during sexual maturation and aging has not been studied in dogs. Such basic information about testicular steroidogenesis–related factors is important from a physiological point of view and for etiological analyses of male hormonal disorders. Thus, the present study examined testicular mRNA levels of the steroidogenic enzymes as well as StAR, COX-1, and COX-2 in prepubertal, postpubertal, and aging dogs.

2. Materials and methods

2.1. Animals

Testicular tissues were obtained from the dogs referred to the veterinary clinic of Osaka Prefecture University (Japan) and other animal clinics outside the university for routine castration. The animals were neither operated on nor killed for this study. Although some older dogs had benign prostatic hyperplasia, all the dogs used had no other reproductive diseases, such as cryptorchidism or testicular abnormality. Table 1 shows the ages, body weights, and breeds of the dogs used for the determination of testicular mRNA levels in prepubertal and postpubertal periods. The relationship between age and testicular mRNA level during aging was analyzed using 44 dogs of different breeds that were aged 1 to 18 years. The ages were corrected by the method of Peters et al. [19], which is modified from Goldston [24] and based on the life expectancy of large dog breeds being shorter than that of small dog breeds. The age of each dog at the time of castration was expressed as a percentage of the geriatric age based on the dog's breed: 11.5 years for small dogs (0–9 kg); 10.9 years for medium dogs (10–23 kg); and 8.9 years for large dogs (24–40 kg). Table 2 shows the geriatric age, sample numbers, and age ranges of the dogs used to determine testicular mRNA levels. The following breeds (and the number of each breed), which included the 1-year-old dogs shown in Table 1, were used: Shih Tzu (9), Miniature Dachshund (5), Yorkshire or Boston Terrier (5), Toy Poodle (4), Chihuahua (3), Mix (3), Golden or Labrador Retriever (2), Shiba Inu (2), Bulldog (1), Cavalier (1), Cocker Spaniel (1), Maltese (1), Poodle (1), Welsh Corgi (1), Pomeranian (1), Dachshund (1), Samoyed (1), Papillion (1), and unknown (1). A part of the testis, including the middle portion was immersed in RNAlater (Qiagen, Valencia, CA, USA) at 4 °C for 24 hours and kept at −80 °C until the extraction of RNA. Some RNA samples used in our previous report [23] were reused (four and six samples in Tables 1 and 2, respectively).

Table 1
Age, body weights, and breeds used for the determination of testicular gene expression in prepubertal (4–6 mo of age) and postpubertal (1 y of age) dogs.

Sample no.	Age	Body weight (kg)	Breed
1[a]	4 mo	4.2	Shih Tzu
2[a]	5 mo	11.3	Mix
3[a]	5 mo	2.0	Chihuahua
4	6 mo	8.6	Shiba
5	6 mo	2.9	M.Dachshund
6	6 mo	2.1	Toy Poodle
7	6 mo	22.7	Golden Retriever
8	6 mo	2.4	Toy Poodle
9	6 mo	4.0	Toy Poodle
10	6 mo	11.9	Mix
11	1 y	2.0	Chihuahua
12	1 y	4.2	M.Dachshund
13[a]	1 y	31.0	Golden Retriever
14	1 y	3.3	Toy Poodle
15	1 y	3.6	Yorkshire Terrier
16	1 y	13.0	Boston Terrier
17	1 y	3.6	Toy Poodle
18	1 y	25.3	Bulldog

[a] The same sample used in our previous report (Tamada et al., 2016 [23]).

2.2. Extraction of RNA and reverse transcription (RT)

Total RNA was extracted using acid guanidinium thiocyanate-phenol-chloroform [25] and quantified by UV absorption measurements. The purity of RNA was evaluated by the ratio of the absorption at a wavelength of 260 nm to that at 280 nm; the values were greater than 1.5 in all samples. The RNA was reverse transcribed into cDNA using a PrimeScript RT reagent kit (Takara, Otsu, Japan) according to the manufacturer's instructions. The starting material was 100 ng of total RNA, and the RT was primed

Table 2
Weight category, corresponding age at which dogs are considered geriatric, and numbers of dogs with normal testes used for the determination of testicular mRNA levels during aging and their age ranges.

Weight category	Geriatric age (y)	No. of dogs	Age range (y)
Small dogs (0–9 kg)	11.5	35	1–12
Medium dogs (10–23 kg)	10.9	5	1–18
Large dogs (24–40 kg)	8.9	4	1–9

The same samples from five small dogs, one medium dog, and one large dog were used in our previous report (Tamada et al., 2016 [23]). The corresponding age at which dogs were considered geriatric is based on Peters et al., 2000 [19].