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Expression of cyclooxygenase-2 in the canine lower urinary tract with regard to the effects of gonadal status and gender

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Abstract

As pituitary gonadotrophins can induce prostaglandin (PG) synthesis and receptors for LH and FSH are present in the canine lower urinary tract (LUT), the objectives of this study were to (i) investigate the expression of COX-2, a key rate-limiting enzyme in PG production, in the canine LUT and (ii) determine if COX-2 expression differs between gender, gonadal status (intact and gonadectomised) and LUT regions. Four regions (body and neck of the bladder as well as proximal and distal urethra) of the LUT were obtained from 20 clinically healthy dogs (5 intact males, 5 intact anoestrous females, 4 castrated males, 6 spayed females). *In situ* hybridization and immunohistochemistry were performed to determine the presence of COX-2 mRNA and protein, respectively. The mRNA and protein expression was semi-quantitatively assessed. The scoring system combined both the distribution and intensity of positive staining and was carried out separately on the three tissue layers (epithelium, sub-epithelial stroma and muscle) for each of four regions of the LUT. In comparison to intact dogs, lower expression ($P < 0.001$) of COX-2 and its mRNA in gonadectomised males and females was observed in all tissue layers of each region of the LUT except in the distal urethra where there was no difference in mRNA expression between gonadal statuses. Regardless of region and tissue layer, intact females expressed more ($P < 0.05$) COX-2 and its mRNA than intact males. However, in gonadectomised dogs, mRNA expression of COX-2 did not differ between genders; males had higher ($P < 0.001$) protein level of COX-2 compared to females. In conclusion, both COX-2 and its mRNA were expressed in the canine LUT and COX-2-regulated PG synthesis in the canine LUT may differ between gonadal statuses and genders. The lower expression of COX-2 in gonadectomised dogs may impair normal function of the LUT and probably implicated in the development of neutering-induced urinary incontinence in the dog.

Keywords: COX-2; Lower urinary tract; Spaying; Urinary incontinence; Dog

1. Introduction

Prostaglandins (PGs) have an essential role in the function of the lower urinary tract (LUT) that consists of the urinary bladder and the urethra. PGs are involved in regulation of bladder tone and the modulation of micturition reflexes [1–3] and provide cytoprotective effects on the urinary epithelium, namely urothelium [4–7]. Production of PGs is mediated by cyclooxygenases

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via conversion of arachidonic acid into prostaglandin endoperoxide (PGH_2), the common precursor for the synthesis of all prostanoids [8]. Cyclooxygenase (COX; so-called prostaglandin endoperoxide H synthase) has two isoforms. These are COX-1 and COX-2 both of which are similar in the structure and efficiency of their catalytic site, although they are encoded by separate genes located on different chromosomes [9]. While COX-1 is present in most tissues and associated with regulation of house-keeping functions [10], expression of COX-2 is observed in certain tissues and can be regulated by various stimuli, i.e. cytokines, growth factors, tumor promoters, mechanical strain [9]. Moreover, COX-2-derived PGs are shown to have an important role in various physiological and pathological conditions [11–15].

Retrospective studies have shown a greater prevalence of urinary incontinence in gonadectomised dogs compared to intact animals and spayed females are at particularly high risk [16]. The temporal relationship between neutering and urinary incontinence appears to be a consequence of the hormonal changes that follow the removal of the gonads; however, the role of gonadal hormones and their endocrine imbalances post-neutering in relation to incontinence are not yet fully defined. Although GnRH treatment has been shown to be an effective cure for urinary incontinence in spayed bitches [17,18] and a functional role of gonadotrophins in the canine LUT has been suggested, the underlying mechanism of regulation of LUT function by gonadotrophins remains unknown.

The expression of receptors for luteinizing hormone (LHR) and follicle-stimulating hormone (FSHR) in the canine LUT suggests that the bladder and the urethra are target tissues for the action of gonadotrophins [19–21]. Moreover, the patterns of expression of LHR and FSHR vary between gonadal status (intact or gonadectomised), gender, region of the LUT (body of the bladder, neck of the bladder, proximal urethra and distal urethra) and tissue layers (epithelium, subepithelial stroma and muscle) [20,22,23]. Because COX-2 can be stimulated by gonadotrophins [24,25], it is interesting to know if this variation in LHR and FSHR in the LUT due to gender and gonadal status affect the expression of COX-2.

We hypothesized that the pattern of COX-2 expression in the canine LUT is related to gender and/or the gonadal status. The aims of the present study were to (i) investigate if COX-2 and its mRNA are present in the canine LUT tissues and (ii) determine if the expression of COX-2 differs between genders and between gonadal statuses.

2. Materials and methods

2.1. Animals and tissue collection

All processes involving the use of animals were carried out under the charity's ethically approved guidelines—the animals were euthanized with an overdose of pentobarbital sodium as they were deemed non-rehomeable for various logistic reasons unrelated to physical health. Twenty clinically healthy dogs free from LUT diseases or any abnormalities of the LUT as determined by clinical and post-mortem examination were included in this study. Dog breeds included Beagle, English bull terrier, Staffordshire bull terrier, Thai ridgeback breeds as well as indeterminate cross breeds. The dogs weighed between 13.0 and 22.5 kg (14.8 ± 0.5; mean $\pm$ S.E.M.). The age of dogs varied between 1 and 5 years (2.3 ± 0.2). The animals were assigned to 4 groups based on their gender and gonadal status: Group 1, intact males ($n = 5$); Group 2, intact anoestrous females ($n = 5$); Group 3, castrated males ($n = 4$); Group 4, spayed females ($n = 6$). The absence of ovarian follicles and corpora lutea determined by post-mortem examination was taken as criteria for anoestrus condition. The time interval between neutering and tissue sampling ranged from 24 to 40 weeks (37.3 ± 2.5).

Tissue collection and preservation were as previously described [20,23]. In brief, LUT tissues were divided into four regions, i.e. the body and neck of bladder as well as the proximal and distal urethras. Formalin-fixed tissues were paraffin-embedded. Sections of 7 μm thickness were cut and applied onto slides (Superforst® Plus; BDH Laboratory Supplies, Poole, Dorset, UK) and left to dry in a warm incubator at 37 °C for 24 h.

2.2. In situ hybridization

The expression of mRNA was assessed using digoxigenin-11-UTP labelled sense and antisense riboprobes as described in our previous studies [20,23]. Both probes for COX-2 were synthesised using ovine COX-2 cDNA, kindly supplied by Dr. Claire Kershaw-Young [26]. Both of the probes were generated using the MEGAscript™ High Yield Transcription T7 and SP6 Kits (Ambion Ltd., Cambridgeshire, UK) following the manufacturer's recommendations. The sequence of ovine COX-2 cDNA (accession number U68486; NCBI, NIH) has 89% homology with the respective canine DNA sequence (accession number AY044905; NCBI, NIH). *In situ*