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Expression of prostaglandin E_2 receptor subtypes in the canine lower urinary tract varies according to the gonadal status and gender

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Abstract

Locally-synthesised prostaglandin E_2 (PGE_2) is pivotal for the function of the lower urinary tract (LUT). This study aimed at investigating the expression and distribution pattern of the four PGE_2 receptor (EP) subtypes in the LUT of intact and gonadectomised male and female dogs. Expression for EP1, EP2, EP3, and EP4 and their mRNA (EP2, EP3, and EP4) was investigated. Twenty clinically healthy dogs were allotted into 4 groups based on their gonadal status and gender including 5 intact males, 5 anoestrous females, 4 castrated males, and 6 spayed females. *In situ* hybridization and immunohistochemistry showed variation in the expression of mRNA and protein for the EP subtypes among tissue layers (epithelium, sub-epithelial stroma, and muscle), regions (body and neck of the bladder as well as proximal and distal urethra) and between gonadal statuses and genders. The expression for the four EPs was intense in the luminal epithelium, intermediate to low in the muscle and the sub-epithelial stroma regardless of gonadal status or gender. Higher expression of all EPs and their mRNAs was observed in the proximal urethra compared to other regions in intact dogs. However, in gonadectomised dogs, the expression did not differ among different regions and was generally lower than in intact dogs particularly in the proximal urethra. Differences in the expression between genders were found and depended on EP subtypes. In conclusion, the results have shown that four subtypes of EP receptors and their mRNAs are present in the canine LUT and their expression was affected by the gonadal status and the gender. The results lead to suggest that an impaired LUT function post-neutering may partly be associated with differences in PGE_2 receptor expression between intact and gonadectomised dogs.

Keywords: EP receptor; Urinary bladder; Urethra; Spaying; Urinary incontinence

1. Introduction

Prostaglandins (PGs) are tissue hormones that maintain local homeostasis [1]. These compounds are synthesized via the cyclooxygenase (COX) pathway by most cell types in response to various hormonal and physiological stimuli. Among the prostanoids, PGE_2 is the predominant PG in the bladder of most mammals [2,3]. The actions of PGE_2 in target cells are mediated through its interaction with a G protein-coupled receptor located on the plasma membrane. Four subtypes of prostaglandin E_2 receptor (EP) (EP1, EP2, EP3, and EP4) have been identified [4]. Functionally, EP2 and

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EP4 are relaxant receptors which mediate an increase in cAMP through the Gs class of G proteins to induce smooth muscle relaxation, while EP1, a contractile receptor that induces calcium mobilization to cause smooth muscle contraction. The EP3 subtype is inhibitory receptor acting through the Gi class of G proteins to cause smooth muscle relaxation via a decrease in cAMP [5].

The involvement of PGs in the physiology of the lower urinary tract (LUT) was first proposed by Gilmore and Vane in 1971 [6] who demonstrated elevated circulating PG-like materials when the bladder of anesthetized dogs was distended with fluid. The importance of PGs as local modulators of the function of the LUT has been investigated accordingly, and the results show that the locally-synthesized PGs can regulate spontaneous bladder contraction and tone, modulate the micturition reflex [7–9] and provide a cytoprotective effect on the urothelium [10–12]. The PG production and the relative amounts synthesized and released by the bladder are species-related [2]. In dogs, increased plasma PGE_2 and $PGF_{2\alpha}$ were detected following a rise of intra-vesicle pressure with a greater rate of increase in the PGE_2 content of the bladder wall [12]. Although it is generally known that PGE_2 has physiological significance in the canine LUT (contraction of the bladder and relaxation of the urethra) [8,9], the expression and distribution of PGE_2 receptors in the bladder and urethra has not yet been reported.

Structural and functional changes in the LUT take place following gonadectomy [13–15]. A close relationship between neutering and the development of canine urinary incontinence particularly in the female dog has been reported [16–18] and the condition has been suggested to associate with changes in hormonal milieu post-neutering, i.e., decreased gonadal-derived hormones and increased LH and FSH [19,20]. The successful use of long-acting GnRH analogues to treat incontinent spayed bitches strongly suggests a role of LH and/or FSH in regulating the function of the canine LUT [20]. However, the role of gonadotrophins in regulating the LUT function remains unknown. Expression of LH and FSH receptors [21–23] and COX-2 [24] in the LUT of dogs has been reported to vary according to their gender and gonadal status (intact or gonadectomised).

It is possible that the variation in the expression of COX-2 in the LUT in relation to the gonadal status and gender [24] may affect the rate of PGE_2 synthesis as well as the pattern of expression for the PGE_2 receptors, resulting in altered tissue integrity and functionality in the LUT of gonadectomised dogs. Thus the aim of the present study was to determine which, if any of the PGE_2 receptors subtypes (EP1, EP2, EP3, and EP4) are present in the LUT of dogs and whether their patterns of expression differed with the gonadal status and/or the gender.

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 their physical health.

The details of animals and tissue collection were the same as described previously in our studies [21,22,24,25]. Briefly, 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. Dogs weighed between 13.0 and 22.5 kg (14.8 ± 0.5; mean ± S.E.M) with a mean age of 2.3 ± 0.2 years (range 1–5 years). Dog breeds included Beagle, English bull terrier, Staffordshire bull terrier, Thai ridgeback, and cross-bred. Twenty dogs were assigned to 4 groups depending on their gonadal status and gender: 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). Anoestrous stage of reproductive cycle in intact females was determined on the basis of an absence of ovarian follicles and corpora lutea at the time of tissue collection. Time interval between neutering and tissue sampling ranged from 24 to 40 weeks (37.3 ± 2.5). Soon after collection, LUT tissues were divided into 4 regions; (i) body of the bladder, (ii) neck of the bladder, (iii) the proximal urethra, and (iv) the distal urethra. The formalin-fixed tissues were paraffin-

Table 1
Oligonucleotide primer sequence and expected amplicon size used for PCR assays.

Gene	Sequences (5′ to 3′)	Product length
EP1		
Forward	CCC AGC ACC TGA CAT GAG T	389 bp
Reverse	ACA GGC CGA AGA AGA CCA T	
EP3		
Forward	CGC TGC TGA TAA TGA TGT TGA	313 bp
Reverse	GAA GAA GCA GGT TCC CTG TG	