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ScienceDirect
Theriogenology 71 (2009) 239–247
Theriogenology
www.theriojournal.com

Neutering affects mRNA expression levels for the LH- and GnRH-receptors in the canine urinary bladder

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Received 9 November 2007; received in revised form 17 June 2008; accepted 18 June 2008

Abstract

Neutering a bitch increases the incidence of acquired urinary incontinence (AUI) 20-fold. Mechanistically this effect is thought to be related to altered steroid/reproductive hormone concentrations and a recent study showed that gonadotrophin releasing hormone (GnRH) analogue treatment improved continence in bitches with AUI. The aim of this study was to examine mRNA expression levels for luteinizing hormone (LH)- and GnRH-receptors in the canine bladder and the correlation between these and *in vitro* contractility of the bladder using age matched entire and neutered, male and female canines and canines with AUI.

Biopsies from the dome of the bladder were dissected post mortem with informed owner consent. mRNA expression for LH- and GnRH-receptor was quantified by rtPCR (relative to β-actin). Contractility was assessed (cumulative concentration response curve for carbachol) in strips of bladder muscle using standard protocols.

Analysis of variance (Tukey post-test) demonstrated thet neutering was associated with significantly increased levels of expression of LH- and GnRH-receptor mRNA in both sexes ($P < 0.01$). mRNA expression for both receptors was significantly higher in female versus male canines. Neither effect was affected by animals' age and/or weight. A significant inverse correlation (Spearman's test) was found between bladder contractility and mRNA expression for both receptors. This effect was most pronounced in canines with AUI which demonstrated the highest mRNA expression levels yet had the lowest contractility of all animals studied. This suggests that increased LH- and GnRH-receptor mRNA expression is associated with changes in bladder function that increase an animal's predisposition to develop AUI.

Keywords: Urinary incontinence; Bladder; Bitch; LH; GnRH; mRNA

1. Introduction

Acquired urinary incontinence (AUI) is a debilitating and so far incurable condition that causes significant welfare problems. Two diverse populations have been identified that are particularly susceptible to AUI, post-menopausal women and spayed female canines. Information relating to the exact pathophysiology of the condition in these two groups is not understood well enough to allow determination of commonality of cause; however, both groups are subject to a reduction in gonadal steroids and associated endocrine changes.

Urinary incontinence (UI) is defined as the involuntary leakage of urine [1] and, in the bitch, is an increasingly recognised clinical problem with severe welfare implications that can lead to the ultimate

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0093-691X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.06.093

euthanasia of the animal. Interestingly, AUI is reported to affect up to 20% of neutered bitches [2] but is seen in less than 1% of intact bitches [3] and is rarely reported in male canines regardless of gonadal status. A direct relationship between neutering and AUI has been reported [4] which is proposed to occur as a consequence of hormonal, vascular or neurological changes [5], rather than mechanical damage of the lower urinary tract, sustained during surgery [6]. Incontinence in the neutered bitch is frequently associated with a decrease in maximal urethral closure pressure [7,8], however, as this is not a defining characteristic of AUI, multiple causative factors are likely to be involved, not all of which will be a consequence of gonadectomy. It has thus been hypothesized that factors in addition to those that decrease urethral closure pressure, lead to a bitch's developing urinary incontinence after neutering. Previous *in vitro* studies have shown that neutering a canine of either gender leads to a decrease in maximal contractile response of the detrusor muscle to both muscarinic and electrical field stimulation as well as a decreased sensitivity to muscarinic stimulation [9]. These decreases in contractile function *in vitro* are similar to those reported in post-menopausal women who suffer from UI due to impaired contractility of the bladder [10].

Given the commonality of endocrine change in the two populations identified as being susceptible to AUI, it is now a widely held hypothesis that the condition may be due to, or be exacerbated by, hormone-mediated effects. This view is supported by the fact that a pharmacological treatment for AUI in the bitch, is steroid replacement [11]. In both spayed bitches and post-menopausal women there is a deficiency of endogenous gonadal steroid hormones which decreases or removes the normal negative feedback to the hypothalamo-pituitary axis, and results in greatly increased production and secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus, and increased secretion of the pituitary gonadotrophins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) [12–18]. This increase in plasma concentrations of LH and FSH post-neutering has been demonstrated to have a direct relationship with the development of urinary incontinence in the bitch [19]. A recent paper has described the clinical use of GnRH analogues to decrease LH and FSH concentrations in neutered female canines suffering from AUI. The results of this study also indicated that GnRH analogue treatment resulted in clinical continence, or a decrease in incontinence, during the treatment period [12,13]. This result would suggest that the increase in the concentrations of either GnRH and/or the pituitary gonadotrophins could be a causative factor for AUI in the bitch. Whilst GnRH and the gonadotrophins are classically thought of as hormones of the hypothalamo-pituitary-gonadal axis, the presence of their receptors in tissues such as skin [20], reproductive tract, prostate and mammary gland [21,22] as well as the urinary bladder [23–25] would suggest more widespread actions. In particular the presence of receptors within the urinary tract would indicate that altered concentrations of gonadotrophins could induce local effects within the bladder which could affect function. As specific tissue action and function rely upon the presence of specific receptors in the tissue it is possible that changes in either absolute receptor numbers or receptor–ligand concentration could influence the action of a tissue. In this regard a study in women has reported a decrease in LH-receptor numbers in the bladder of post- compared to pre-menopausal women [25], and a number of recent studies in the canine have reported either a decrease [24,26] or no effect on LH-receptor mRNA concentrations after neutering [17]. However, there are no published reports of mRNA levels for LH-, FSH- or GnRH-receptors in the bladder of bitches known to be suffering from AUI or the correlation between detrusor contractility, a factor known to be involved in UI [10,27], and receptor mRNA expression levels within the urinary bladder.

The aim of this study was to determine the effects of gonadal status and gender on the expression levels of GnRH-, LH- and FSH-receptor mRNA in the canine urinary bladder and to investigate whether these levels are altered in individuals suffering from AUI. A further aim was to see if there is a correlation between the expression of the mRNAs for these receptors and maximal contractility of the bladder, using age matched groups of entire and neutered, male and female canines.

2. Materials and methods

2.1. Animals and tissues

The study was approved by The University of Glasgow Veterinary School's ethical review committee. A total of 78 canines were included in the study, with a mean age of 6.0 years (range 1–16 years) and a mean weight of 22.7 kg (range 8–50 kg). The majority of canines were cross bred, with no pedigree breeds appearing more than once.

In all cases tissue was collected from canines euthanized (intravenous overdose of pentobarbatone),