SYMPATHETIC INNERVATION OF VESTIGIAL UTERUS OF THE EUROPEAN BISON BULLS

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Abstract

This study describes the expression of DßH as a marker of sympathetic (catecholaminergic) innervation in the vestigial uterus of adult European bison (Eb) bulls. Cryo-sectioned uteri were examined by fluorescent immunohistochemistry with the use of primary rabbit DßH polyclonal antibodies. The DßH-immunocomplexes (antigens/polyclonals) were visualised by secondary donkey anti-rabbit biotinylated IgG, and then with cyanine (CY™3)-conjugated streptavidin. The DßH immunodetection with CY™3 for the Eb uterine sections was performed in parallel to a positive control (porcine sympathetic paravertebral ganglion), and a negative control (without primary anti-DßH polyclonals). This is the first paper describing the identification of DßH expression within the vestigial uterus of the adult Eb bulls. The distribution pattern of DßH expression localised within immunoreactive (IR) uterine nerve fibres of the bulls resembled that in the control female uterus. The DßH-IR nerve fibres were identified in the entire cross-section of each uterus, with generally higher density in the myometrium than in the endometrium. The ratio of the endometrial/myometrial DßH-expression (percentage of area with IR-signals) was comparable in all males and females (0.78 and 0.64, respectively). However, the DßH-expression area (%) was significantly lower within the male uterine endometrial (P<0.01) and myometrial regions (P<0.001) comparing to the female counterparts. Presumably, the DßH-IR expression within the male Eb uterus is associated with a trophic effect of noradrenaline released by sympathetic nerve fibres influencing nutrient supply of this vestigial organ in the Eb bulls.

Key words: European bison, bull, vestigial uterus, DßH, dopamine β-hydroxylase.

The European bison (Eb), also known as a Wisent, is classified as the endangered taxon in the international Red List of Threatened Species. Our previous proteomic studies on two different bison species were performed with the use of various uterine tissues of adult females, mainly placenta – collected during different pregnancy stages (4, 14). However, genomic screening of the Polish and Swedish Eb populations revealed a considerable diversity of the pregnancy-associated glycoprotein gene family (PAG) between the bulls from both populations (15, 16). The highest genomic diversity of the Polish Eb population (examined by amplicon-length PAG polymorphism) has been found mainly in bulls in which the reproductive system was anatomically abnormal. The observed abnormalities concern frequent existence of the vestigial uterus observed in the Polish Eb bulls (approx. 80%), in which such masculine uterus was detected (2, 13). Unfortunately, similar studies pertaining the vestigial male uterus of the Eb are very limited and cannot be assessed by international data basis searching. Recently, another our study indicated the high diversity in morphology and total protein profiles of vestigial uteri in the Eb bulls (8).

Dopamine β-hydroxylase (DßH; EC 1.14.17.1) converts dopamine to noradrenaline, and is a well-known marker of sympathetic (catecholaminergic) nervous system. Previously, we indicated a specific pregnancy-stage dependent DßH expression in the synepitheliochorial Eb placenta (12). However, so far, the DßH expression has never been examined within the vestigial uteri innervations of any male mammals, and especially in the Eb bulls. The objective of this study was to identify the DßH expression – as the marker of sympathetic innervations of the vestigial uteri in the Eb bulls.

Material and Methods

Animals and uterine tissue collection. Animals were from the Polish populations of the Bialowiesza National Park and the Borecka Forest at North-East
region of Poland. All uterine tissues were collected post mortem from seasonally eliminated adult bulls (n=6; 5–12 years of age) and one juvenile female (6 months of age) – used as appropriate control (due to a lack of main ovarian steroid hormones – oestrogens and progesterone influencing uterus development). The uterus was collected in the agreement with the governmental and the local authorities, immediately frozen, and stored at -20°C until tissue processing.

Specificity of fluorescent DβH-immunodetection. The DβH-immunodetection was performed as described previously (12) with some modification. Briefly, cryosectioned (10 µm) uteri were fixed with buffered 4% paraformaldehyde, blocked with 0.1% bovine serum albumin fraction V, then subjected to heterologous (cross-species) fluorescent immunohistochemistry (F-IHC) with the use of primary rabbit anti-DβH polyclonals (1:8,000; Affiniti-Biomol, UK) raised against bovine DβH antigen. Specificity of primary anti-DβH polyclonals was confirmed previously (by Western blotting of total proteins extracted from the lumbar sympathetic paravertebral ganglia) (14). On the sectioned uterine tissues, the DβH-immunocomplexes were visualised by secondary donkey anti-rabbit biotinylated IgG (1:1,000), and then with cyanine (CY™3)–conjugated streptavidin (0.2 µg/mL), as a fluorophore (Jackson ImmunoResearch, USA). The DβH immunodetection with CY™3 (550 nm excitation/570 nm emission) for the Eb uterine sections was performed in parallel with a positive control (porcine sympathetic paravertebral ganglion) and a negative control (without primary anti-DβH polyclonals) that were similarly treated. The immunoreactive (IR) DβH-CY™3 signals were examined in fluorescent (546–619 nm filters) and bright fields using BX51 microscope equipped with digital camera (Olympus, Japan), then the fluorescent and parallel morphological micrographs were archived (ACDSee v. 7.0; ACD Systems, USA). In addition, the obtained images of the DβH-CY™3 signals within the endometrial and myometrial sections of the male uterus were statistically evaluated by comparison with the appropriate counterparts of the female control (percentage ratio of area with IR-signals) using Cell F Software (Soft Imaging Systems GmbH, Germany). The ratio of the endometrial/myometrial DβH-expression was analysed by one-way ANOVA and the significant differences were marked.

Results

The performed F-IHC revealed the DβH-CY™3 signals within uterine nerve fibres of the Eb bulls, that resembled DβH-distribution pattern localised in the control female uterus (Fig. 1). The DβH-immunoreactive (IR) nerve fibres were detected within the entire uterine sections including: luminal endometrial epithelium and glands (Figs 1 a–d), myometrium (Figs 1 e–f), and blood vessels, mainly arteries (Figs 1 g–j).

![Fig. 1.
The DβH-expression in nerve fibres detected within the vestigial uterus of the European bison bulls, in comparison to uterine structure of juvenile female – used as a control: endometrial epithelium (a, b), glands (c, d), myometrium (e, f) and blood vessels (g–j). The size bar is 200 µm.](image-url)
Fig. 2. Density (%) of DβH-immunoreactive nerve fibers within the vestigial uterus of the European bison males in comparison to control female uterus. ** P≤0.01, *** P≤0.001.

Statistical image evaluation indicated a significantly (P<0.05) increased DβH-IR nerve fibres density in the myometrium in comparison to the endometrium of the bull uteri and control female (Fig. 2). The ratio of the endometrial/myometrial DβH-expression (percentage of area with IR-signals) was comparable in all males and the female (0.78 and 0.64, respectively). However, the DβH expression area of the IR-nerve fibres was significantly lower in the male uterine endometrium (P<0.01) and myometrium (P<0.001) comparing to the equivalent regions of the female uterine counterparts.

Discussion

This is the first paper describing the DβH expression (IR nerve fibres) within the vestigial uterus of the bulls of the endangered Eb. The DβH-IR expression within uterine nerve fibres of the Eb bulls indicates that this vestigial organ is controlled by the sympathetic (catecholaminergic) innervation, similarly as in female uterus.

Our results are very difficult to discuss because of a very limited number of similar studies performed on the vestigial uterus that exists in males of various species. Previously, the vestigial masculine uterus has been observed in a few other mammals: the hamster and beaver (7, 20), horse and donkey (9, 11), dog and cat (10, 19) and the human (3). However, the DβH expression has not been identified yet in any vestigial male uterus of these mammals.

Previous studies only revealed that noradrenaline (NA) can directly stimulate the glandular secretion and steroidogenic activity in the female genital tract of the guinea-pig (18). The postganglionic sympathetic nerve fibers may release NA together with other neuropeptides in the bovine female uterus (5), to control vasomotor and myometral uterine activity through activation of various receptor subtypes (1, 6). All previously described data indicate NA as one of the most important trophic factors regulating nutritional supply to the uterus. However, to obtain the evidence of a potential DβH role in the reproductive system of each mammalian species requires producing (by gene targeting) a knockout model with catecholamine deficiency (17). Thus in the future, at least some animals lacking DβH, and therefore unable to synthesise the NA or adrenaline are required for a conclusive elucidation of the DβH expression in the females, as well as in the bulls with the vestigial uterus.

In conclusion, presumably, the DβH expression in the masculine Eb uterus is associated with a trophic effect of NA released by the catecholaminergic nerve fibres (originating from the autonomic sympathetic ganglia) influencing nutritional supply of this vestigial organ in the bulls of the Eb, in which seasonal reproductiveness is presently expected to be decreased. Therefore, a potential role of the DβH expression and its specific regulation within the vestigial masculine uterus of the endangered Eb taxon requires further investigations.

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References


