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Seasonal reproductive activity and innervation of vas deferens and accessory male genital glands in the water buffalo (*Bubalus bubalis*)

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ABSTRACT: Autonomic nerves supplying mammalian male internal genital organs have an important role in the regulation of reproductive function. To find out the relationships between the neurochemical content of these nerves and the reproductive activity, we performed an immunohistochemical study in a species, the water buffalo, exhibiting a seasonal sexual behaviour. The distribution of noradrenergic and peptide-containing nerves was evaluated during the mating (autumn-winter) and non-mating (spring-summer) periods. During the mating period, a dense noradrenergic innervation was observed to supply the vas deferens as well as the accessory genital glands. Peptide-containing nerves were also observed but with a lower density. During the non-mating period noradrenergic nerves dramatically reduced. These results suggest that there is a neuro-endocrine interaction between androgen hormones and the autonomic nerve supply in the regulation of male water buffalo reproductive functions.

Key words: Testosterone, Pelvic ganglia, Neuropeptides.

INTRODUCTION - The innervation of mammalian male internal genital organs originates from different autonomic ganglia, i.e. pelvic, inferior mesenteric and sympathetic chain ganglia as well as from dorsal root ganglia (Kaleczyc et al., 1993; Danuser et al., 1997) and has an important role in the regulation of reproductive function. Morphological and neurochemical characteristics of nerve fibres supplying vas deferens and accessory genital glands have been well documented in humans as well as in laboratory (Kepper et al., 1995) and domestic mammals (Arrighi et al., 1997). Few data, however, are available regarding the neurotransmitter profile of these fibres in species exhibiting a seasonal sexual activity. Mature (over 4 years old) male water buffaloes are known for their poor reproduction rate. They, indeed, show a seasonality in the sexual activity characterized by a mating season during the autumn–winter and a long non-mating season from the late spring to the beginning of the autumn. These two seasons are related with changing levels of circulating sexual hormones and changing hypophyseal responses to luteinizig hormone–releasing hormone (LH–RH) administration tests (Zicarelli et al., 1981). Information regarding the relationships between the neurochemical characteristics of the nerve supply of male buffalo genital organs and the seasonality of the sexual activity could be of interest in the explanation of neuroendocrine mechanisms which regulate the male reproduction in mammals.

MATERIAL AND METHODS - This study was performed on mature male water buffaloes (aged 5 years). Fresh segments of vas deferens, ampulla deferentis, vesicular gland and the disseminated portion (the only existing) of the prostate were collected immediately after slaughter and immersed in fixative. After fixation, the samples were embedded in 100% OCT. Coronal and sagittal sections of 10–20 micron thickness were cut. The frozen sections were immunostained according to the fluorescent technique. The primary antibodies were: PGP 9.5, TH, VIP, NPY.

RESULTS AND CONCLUSIONS - During the autumn–winter, the overall distribution of the innervation was studied using PGP 9.5. All the investigated tissues were abundantly innervated. In the vas deferens, a very dense nerve network was distributed both in the muscle coat and lamina propria (Figure 1). In the ampulla deferentis and vesicular gland, many positive fibres were found to innervate the muscle coat as well as the stromal smooth muscle and the adenomeres. In the prostate gland, a dense network of nerve fibres was observed within the muscle coat and around the gland adenomeres.

In the vas deferens, ampulla and vesicular gland, the distribution of TH-positive fibres almost completely matched that of PGP 9.5. In the prostate, large TH-positive nerves were found in the adventitial layer and a dense network of positive fibres was observed within the muscle coat. In all the organs, most of the NPY-positive fibres which were located within the muscle coat and around blood vessels were also TH-positive. In the lamina propria of the vas deferens and within the prostatic glandular tissue, the majority of NPY-positive fibres also expressed VIP. VIP-positive fibres were observed to form a loose network in the lamina propria of the vas deferens. In the accessory glands, these fibres were found to innervate the stromal muscular tissue and adenomeres. In addition, many of the VIP-positive fibres innervating the lamina propria of the vas deferens and the prostate gland and, at least a part of those innervating the ampulla and vesicular glands, were also NPY-positive.

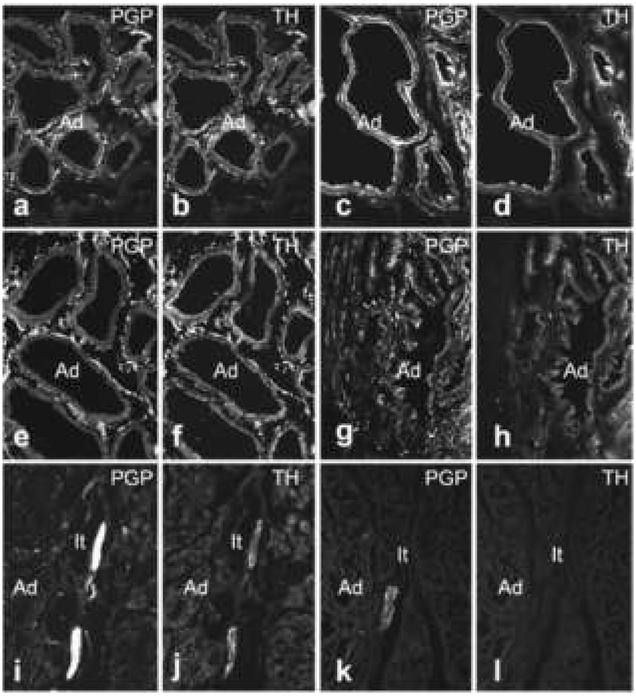
During the spring–summer, the general distribution pattern of the innervation was similar to that observed in the autumn–winter. TH-positive fibres dramatically reduced during the spring–summer in the vas deferens (Figure 1) as well as in the ampulla and vesicular gland (Figure 2e–h). Except the subepithelial zone of the vas deferens, the density of PGP 9.5- and TH-positive axons was similar in the autumn–winter (Figures 1a–d). In spring–summer, PGP 9.5-positive axons were unchanged whereas TH staining was much decreased (Figures 1e–g). In the prostate, a reduction of TH-positive fibres was observed in the nerves running within the muscle coat and interglandular septa. NPY- and VIP-positive fibres clearly reduced during the spring–summer in the vas deferens as well as in the accessory glands.

The present study has shown that the buffalo vas deferens and accessory genital glands are richly innervated by autonomic nerves. Important seasonal changes, however, have been observed regarding the neurochemical content of the nerve fibres. These changes primarily involved the catecholaminergic nerves.

During the autumn–winter, catecholaminergic fibres represent the most common population of fibres supplying the vas deferens and accessory genital glands. Because both catecholamine-synthesising enzymes studied, TH and DBH, coexisted within catecholaminergic

nerves, these nerves should be considered as noradrenergic. During the spring–summer, noradrenergic fibres almost completely disappeared, thus, suggesting an interaction between noradrenergic innervation and sexual hormones. Noradrenergic innervation supplying the buffalo ductus deferens and accessory glands seems to be, therefore, highly androgen-sensitive and its integrity during the mating season is probably essential in the maintenance of many reproductive functions.
During the spring–summer neuropeptides-positive fibres clearly reduced, thus, suggesting that peptides are positively regulated by androgens in the neurons which supply buffalo male genital organs. In conclusion, the present study has revealed the presence of distinct populations of nerves supplying the vas deferens and accessory genital glands of the water buffalo. The neurochemical characteristics of these nerves showed some differences if compared with those described in other mammalian species. In addition, important changes in these characteristics occurred during the season in which buffaloes decrease their reproductive activity. The immunoreactivity to neuropeptides were significantly less intense during this period, thus, suggesting the presence of complex interactions between androgen hormones and the autonomic neurons in the regulation of male water buffalo reproductive functions.

Figure 1. PGP/TH double staining of the vas deferens: comparison between (a-d) the mating and (e-h) non mating seasons. bar= 100 micron. Mc: muscle coat; Lp: lamina propria.



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