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An immunohistochemical survey of peptide-containing nerves in the copulatory apparatus of several male birds

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Summary. Distribution of peptide-containing nerves in male copulatory apparatus as well as rectal and cloacal ganglia were examined immunohistochemically in cock, guinea fowl, duck and muscovy duck. Neurofilament protein-immunoreactive nerves were also examined to investigate general distribution of nerves.

Substance P (SP)-, vasoactive intestinal polypeptide (VIP)-, calcitonin gene-related peptide (CGRP)-, and neuropeptide Y (NPY)-immunoreactive (IR) nerves were detected in all four species of birds whereas leucine enkephalin (LENK)-IR nerves were detected only in duck and muscovy duck. In the rectal and cloacal ganglia, peptide-IR nerves were detected in varying degrees of abundance. Most noticeable distribution was found in the vascular body, where SP-IR nerve fibres were more abundant in duck and muscovy duck, VIP-IR fibres in muscovy duck and CGRP-IR fibres in duck, than in other regions and other species, respectively. A few peptide-IR nerve fibres were detected in other cloacal regions, although a peculiar pattern in distribution unique to a certain region could be not found.

The present results suggest that the peptide-containing nerves participate in the regulation of tumescence, erection and detumescence of male copulatory apparatus of birds and their regulatory mechanisms are different depending on the species.

Key words: Immunohistochemistry, Nerves, Copulatory organ, Aves

Introduction

The mechanism in tumescence, erection and detumescence of male copulatory organ is different in birds from mammals (King, 1975). An essential part of the mechanism leading to the erection in male birds is the influx of lymph into the copulatory apparatus, phallus or phallic body. Paracloacal vascular body is thought to play principal roles in the production of the lymph (Nishiyama, 1955; Nishiyama and Ogawa, 1961), which is likely to be regulated by an autonomic nervous system distributed in the vascular body (Yamano, 1984). The presence and significance of peptide-containing nerves have been confirmed in male genital tract of mammals (see Owman and Stjernquist, 1988). Peptide-containing nerves should also contribute extensively to autonomic innervation of the copulatory apparatus of birds, although information concerning the possible occurrence of peptide-containing nerves in avian genital organ is not available. The aim of the present study is to give immunohistochemical information connecting with possible functions of peptide-containing nerves in the copulatory apparatus of several male birds with special reference to the vascular body. For the comparison, neurofilament protein-immunoreactive nerves were investigated to reveal the general pattern of the innervation.

Materials and methods

Materials were obtained from cock *(Gallus gallus* var *domestica)*, duck *(Anas platyrhyncos* var *domestica)*, muscovy duck *(Cairina moschata)* and guinea fowl *(Numida meleagris)*. Cloacal region was dissected out after bleeding under anaesthesia with sodium pentobarbital and examined for paracloacal vascular body, phallic body or phallus, lymph fold, papilla of ductus deferens, ejaculatory fossa (urodeum), coprodeum, proctodeum, and rectal and cloacal ganglia. These samples were fixed in Bouin's fluid overnight and processed routinely for embedding in paraffin. Sections cut at 5 µm were processed for immunohistochemistry using Vectastain® *Elite* ABC Kit (Vector, Burlingame). Sections were pretreated with methanol containing 0.3% H_2O_2 for blocking endogenous peroxidase activity. For the primary reaction, the following antisera (antibodies) were used: neurofilament protein pooled monoclonal antibodies raised in mouse (Immunotech, Cedex, Ref.

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0168, 1:2,000); substance P monoclonal antibody raised in rat (Sera Lab, Sussex, Lot. B6E35, 1:2,000); vasoactive intestinal polypeptide polyclonal antiserum raised in rabbit (Amersham Int. plc., Amersham, Lot. 7, 1:8,000); calcitonin gene-related peptide polyclonal antiserum raised in rabbit (Amersham, Lot. 2, 1:6,000); neuropeptide Y polyclonal antiserum raised in rabbit (Amersham, Lot. 10, 1:8,000); and leucine enkephalin polyclonal antiserum raised in rabbit (UCB-bioproducts, Brussels, Lot. E2929-4-2A, 1:8,000). Immunoreaction sites were visualized with Tris-HCl buffer solution (pH 7.4) containing diaminobenzidine hydrochloride and H_2O_2. These sections were mounted through the usual steps. No staining was observed in this study if the sections were incubated in normal serum instead of the primary antisera or when one of each step was omitted.

Results

Neurofilament protein-immunoreactive (NFP-IR) nerves

Nerve fibres and nerve cell bodies showed varying intensity of immunoreactivity from negative to very strong. NFP-IR nerve fibres and cell bodies were abundant in the rectal and cloacal ganglia of all species of birds examined (Fig. 1a). NFP-IR nerve fibres were detected in the vascular body of all birds, being relatively abundant in duck and muscovy duck (Fig. 1b). In other regions, a few fibres and cell bodies were

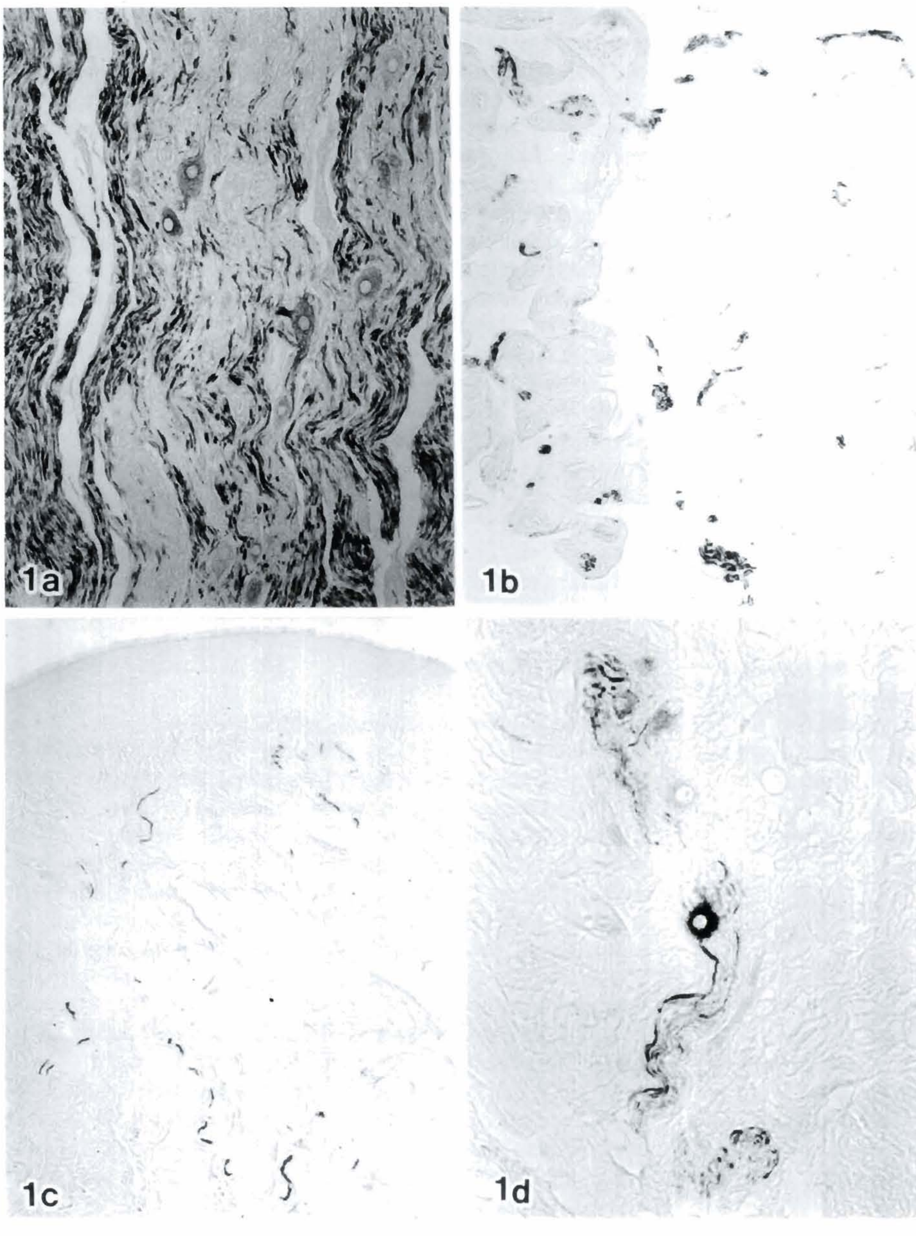

Fig. 1. Neurofilament protein-immunoreactive nerves in the rectal ganglion of cock **(a)**, vascular body of muscovy duck **(b)**, median phallic body of cock **(c)** and myenteric plexus in the coprodeum of muscovy duck **(d)** a-c x 150, d x 300

detected here and there, showing no particular pattern in distribution unique to the certain region and species (Figs. 1c,d).

Substance P-immunoreactive (SP-IR) nerves

SP-IR nerve cell bodies were detected only in the submucosal ganglia of coprodeum. In the rectal and cloacal ganglia of all birds, abundant SP-IR nerve fibres were detected around and among non-reactive nerve cell bodies, showing slightly patchy distribution (Fig. 2a). SP-IR nerve fibres were relatively abundant in the vascular body of duck and muscovy duck, few in guinea fowl and very few in cock (Figs. 2b,c). In other regions, few SP-IR nerve fibres were detected, predominating in the mucosa and muscle layer of coprodeum and proctodeum (Figs. 2d,f). SP-IR nerve fibres were also detected surrounding non-reactive nerve cell bodies located solely in muscle layer and connective tissue space (Fig. 2e).

Vasoactive intestinal polypeptide-immunoreactive (VIP-IR) nerves

A few nerve cell bodies showed weak immunoreactivities for VIP in the rectal and cloacal ganglia only of duck and muscovy duck (Fig. 3a).VIP-IR nerve fibres were abundant in the rectal and cloacal ganglia of duck and muscovy duck while there were few in cock and guinea fowl. They showed patchy distribution in both ganglia of all birds. VIP-IR nerve fibres were detected relatively abundant in the vascular body of muscovy duck, few in duck and the least in cock and guinea fowl (Fig. 3b). In other regions, VIP-IR nerve fibres were detected at random, being relatively more abundant in the mucosa and muscle layer of coprodeum and proctodeum (Figs. 3d-f). VIP-IR nerve cell bodies were also detected in the ganglia in muscle layer of coprodeum of duck and guinea fowl and in the submucosal ganglia of coprodeum of guinea fowl (Fig. 3f). Nerve cell bodies present

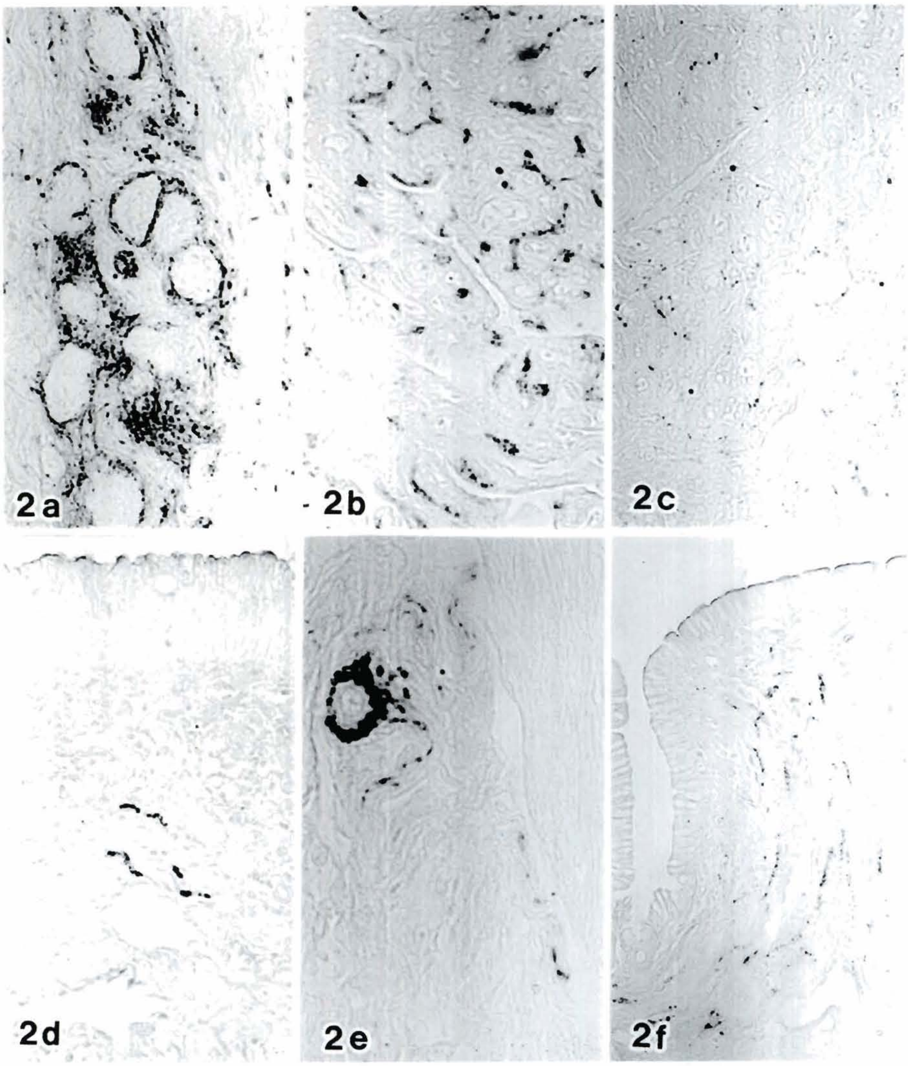

Fig. 2. Substance P-immunoreactive nerve fibres in the cloacal ganglion of cock **(a)**, vascular body of muscovy duck **(b)**, and duck **(c)**, lateral phallic body of cock **(d)**, ejaculatory fossa of guinea fowl **(e)** and the mucosa of coprodeum of cock **(f)**. In a and e, fibres embrace non-reactive nerve cell bodies. a-d x 300, e x 400, f x 150

alone in the connective tissue space showed VIP immunoreactivity at times and some of them were located in the vicinity of arterioles (Figs. 3c,d).

Neuropeptide Y-immunoreactive (NPY-IR) nerves

A few NPY-IR nerve cell bodies were detected only in the rectal and cloacal ganglia of cock, showing weak immunoreactivities (Fig. 4a). NPY-IR nerve fibres were relatively more abundant in both the ganglia of duck and muscovy duck than in cock and guinea fowl, showing patchy distribution in all species (Fig. 4b). NPY-IR nerve fibres were detected sparsely in the vascular body of muscovy duck while they were rare or absent in other species (Fig. 4c). In other regions, NPY-IR nerve fibres were rare and showed unstable occurrence, although they seemed to be relatively more abundant in the muscle layer of coprodeum and proctodeum.

Calcitonin gene-related peptide-immunoreactive (CGRP-IR) nerves

CGRP-IR nerve cell body was not detected. CGRP-IR nerve fibres were detected in the rectal and cloacal ganglia in all birds, showing patchy distribution (Fig. 5a). They were relatively more abundant in cock than in other species, although they seemed to be less numerous than other peptide-IR nerves. CGRP-IR nerve fibres were very rarely detected in the vascular body, showing an unstable occurrence (Fig. 5b). In other regions, CGRP-IR nerve fibres were detected at random and their occurrence was not stable (Fig. 5c).

Leucine enkephalin-immunoreactive (LENK-IR) nerves

LENK-IR nerves were detected in duck and muscovy duck but not in cock and guinea fowl. A few nerve cell bodies showed weak to moderate intensity of LENK immunoreactivity in the rectal and cloacal

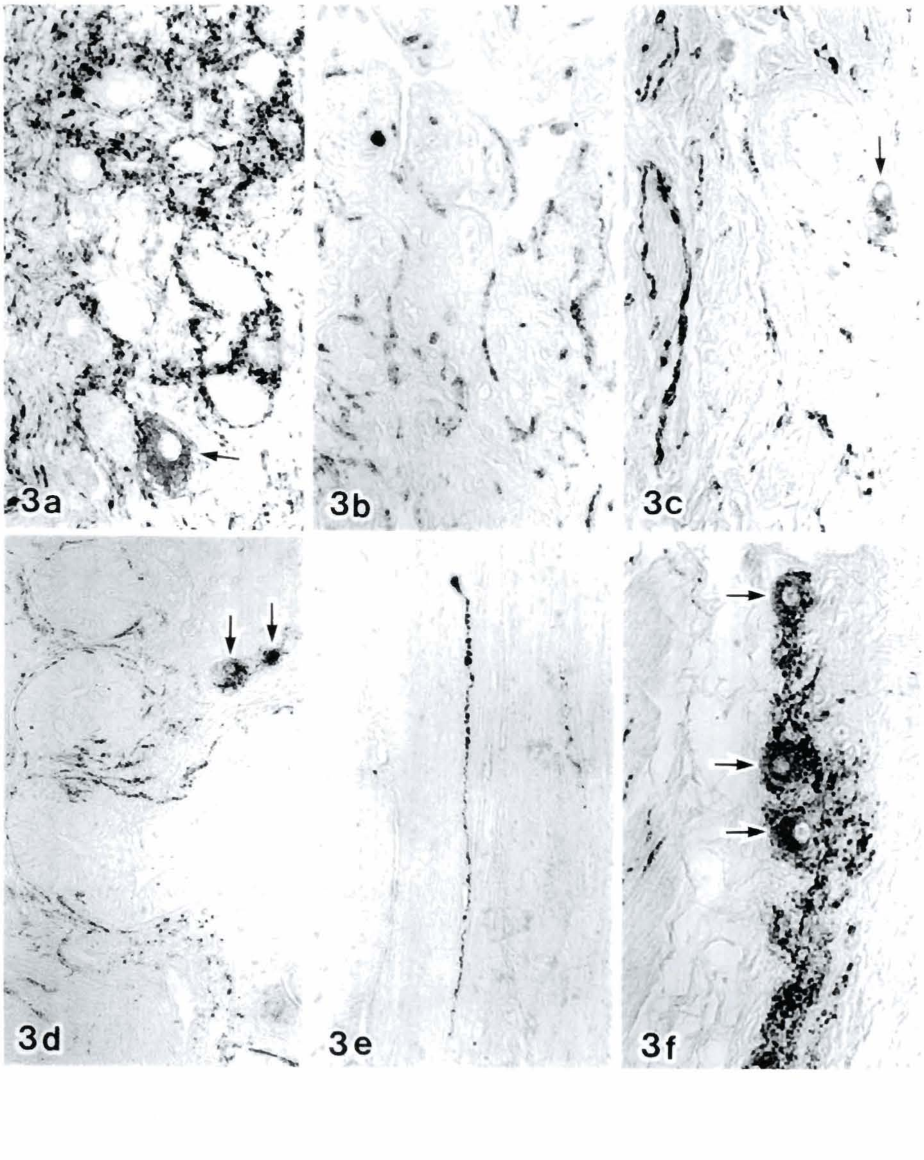

Fig. 3. Vasoactive intestinal polypeptide-immunoreactive nerves in the cloacal ganglion of duck **(a)**, vascular body of muscovy duck **(b)**, papilla ductus deferens of duck **(c)**, mucosa of coprodeum of guinea fowl **(d)**, muscle layer of the ejaculatory fossa of cock **(e)** and the myenteric plexus of coprodeum of duck **(f)**. Arrows indicate immunoreactive nerve cell bodies. a, b, e, f x 300, c, d x 150

ganglia (Fig. 6a). LENK-IR nerve fibres in both ganglia were detected with relative abundance, showing patchy distribution (Fig. 6a). Nerve fibres very rarely showed LENK immunoreactivity in the vascular body (Fig. 6b). In other regions, LENK-IR nerve fibres were few and unstable in occurrence, although they seemed to be relatively abundant in the muscle layer of coprodeum (Fig. 6c).

Discussion

Present immunohistochemical study confirmed the presence of peptide-containing nerves in the copulatory apparatus as well as the rectal and cloacal ganglia, which were immunoreactive for SP, VIP, CGRP, NPY and LENK. The distribution of these nerves is within that of NFP-IR nerves, although specific pattern in distribution unique to a certain region could not be identified in the

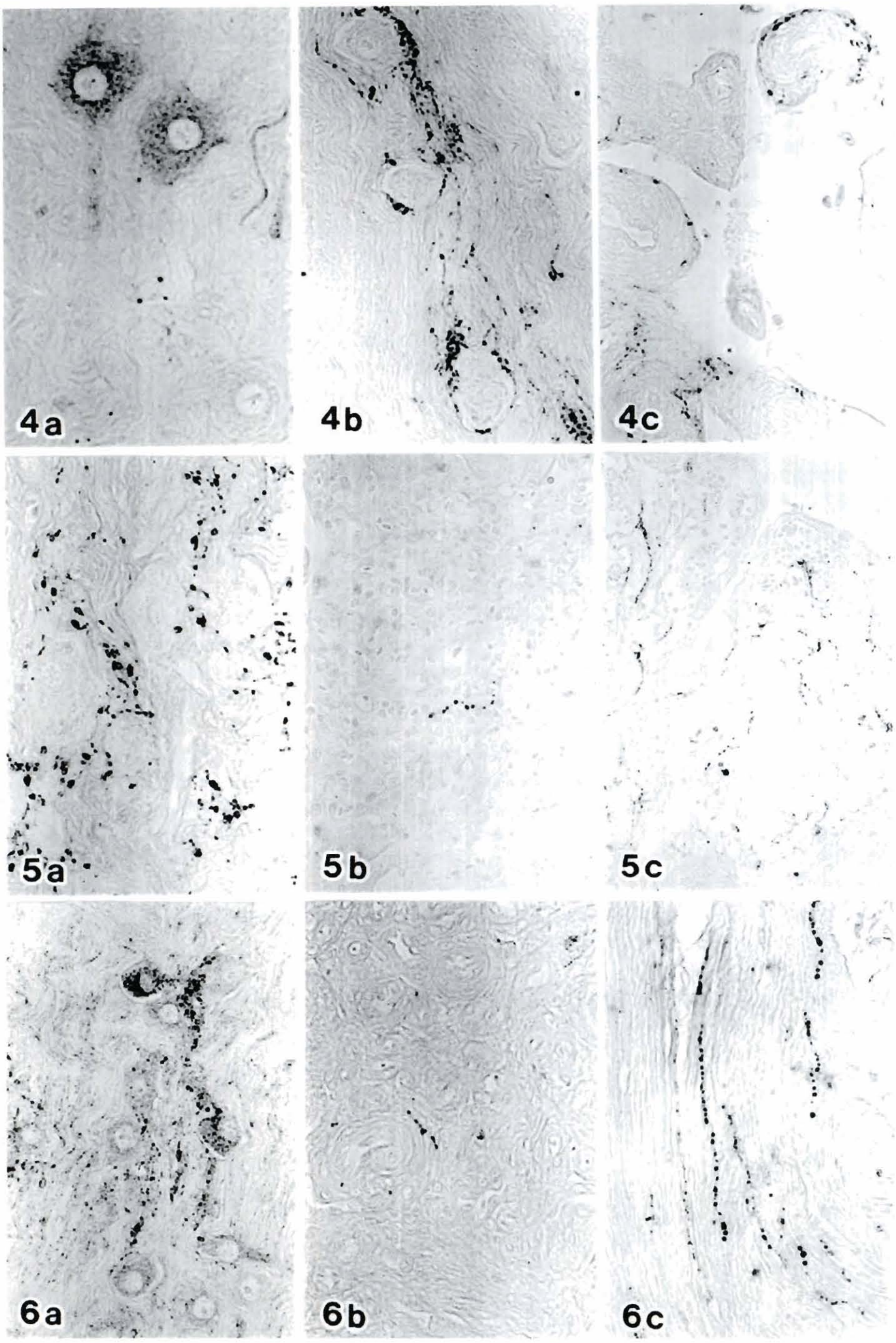

Fig. 4. Neuropeptide Y-immunoreactive nerves in the cloacal ganglion of cock **(a)** and muscovy duck **(b)** and the vascular body of muscovy duck **(c)**. a-c x 300

Fig. 5. Calcitonin gene-related peptide-immunoreactive nerve fibres in the cloacal ganglion of cock **(a)**, vascular body of cock **(b)** and the mucosa of ejaculatory fossa of duck **(c)**. a, b x 300, c x 150

Fig. 6. Leucine enkephalin-immunoreactive nerves in the cloacal ganglion of muscovy duck **(a)**, vascular body of duck **(b)** and muscle layer of coprodeum of duck **(c)**. a-c x 300

present study in the cloacal region except for the vascular body.

Paracloacal region including copulatory organ is assumed to be innervated by both sympathetic and parasympathetic pathways through the rectal and cloacal ganglia and pudendal nerve, although this is not yet fully substantiated (Knight, 1970; Baumel, 1975). Since nerve cell bodies were not detected in the vascular body in the present study, nerve fibres detected in the vascular body were likely to come from other regions such as the rectal and cloacal ganglia and pudendal nerve. That is, they are extrinsic to the vascular body in origin. Meanwhile this body may be innervated by both extrinsic and intrinsic nerves in certain region other than the vascular body, since local ganglia were detected at times in location. It is possible that these local ganglia also innervate the vascular body.

Few nerve fibres were detected in the cloacal region other than in the vascular body. Although functional significance of these nerves is not assumed at present, they should play a role because of their location. The relative abundance of peptide-containing nerves in the vascular body is most noticeable in relation to the lymph production which plays a principal role in the erection of phallus or phallic body. Neuropeptides clarified in the vascular body are assumed to have a regulatory role in the mammalian genital organ. Especially, VIP may play key roles in the penile erection by regulating the blood flow in penis (Juenemann et al., 1987). Although accumulated evidence is yet little, other neuropeptides examined in the present study are also likely to have regulatory roles in the mammalian genitalia (Owman and Stjernquist, 1988). Although the functional role of these neuropeptides in avian copulatory organ is not yet clarified, they are most likely to also regulate the ability of vascular body to produce the lymph. There were inter-species differences in the abundance of peptide-containing nerves in the vascular body, depending on the kind of neuropeptides. That is, SP-IR nerves were relatively more abundant in duck and muscovy duck, VIP-IR nerves in guinea fowl and CGRP in duck. Duck and muscovy duck have intromittent phallus, while cock and guinea fowl have non-intromittent type. It could be assumed that prominent type of peptide-containing nerves innervating the vascular body is associated with the degree of development of phallus. Since prominent type of nerves distributed in the copulatory apparatus seems to be different in birds from mammals, as clarified in the present study, it is possible that the peptidergic regulatory mechanism involved in the copulatory apparatus is different in birds and mammals.

The present results would thus indicate that peptide-containing nerves may have physiological roles in the neurogenic mechanism in the tumescence, erection and detumescence of the copulatory apparatus of birds and that this mechanism may be different depending on the avian species.

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