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Nerve Growth Factor of Horsfield's Shrew, *Crocidura horsfieldi*

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The content of nerve growth factor (NGF) in the submandibular gland of Horsfield's shrew (*Crocidura horsfieldi*; *C. horsfieldi*; Insectivora) showed sexual difference (male > female). Submandibular gland of *C. horsfieldi* is known to express male dominant sexual dimorphism at the striated duct portion and a relationship to the presence of NGF was suspected. *C. horsfieldi* and house musk shrew (*Suncus murinus*; suncus; Insectivora) possessed NGF in the striated duct portion of their submandibular glands. Although suncus has secretory granules in the secretory portion of the submanfibular gland, NGF was not detected at that portion. In the prostate glands of *C. horsfieldi* and suncus, NGF localization was observed at the epithelial cells and in the lumen. These results support our current hypothesis that large amounts of NGF exist in the striated duct portion of the submandibular gland which possesses male dominant sexual dimorphism. Furthermore, the prostatic NGF localization proved to follow the general rule *i.e.* NGF is localized at the epithelial cells and in the lumen.

Keywords——nerve growth factor; Horsfield's shrew (*Crocidura horsfieldi*); house musk shrew (*Suncus murinus*); submandibular gland; prostate gland; immunohistochemistry

Introduction

Nerve growth factor (NGF) ia a polypeptide, which promote the regeneration, function maintenance and survival of the neural crest derived sensory and sympathetic neurons.[1] Nerve growth factor is now thought to be one of the neurotrophic factors.[2] So far, biological sources of NGF have been limited to snake venom, mouse submandibular gland, rabbit and guinea pig prostate glands, bovine seminal plasma, and suncus submandibular and prostate glands.[3] Nishiyama and Saito[4] found NGF in the submandibular gland of big clawed shrew (*Sorex unguiculatus*; Insectivora), submandibular and prostate glands of Horsfield's shrew (*Crocidura horsfieldi*; Insectivora), submandibular gland of Japanese field vole (*Microtus montebelli*; Rodentia), and in the submandibular and prostate glands of field mouse (*Mus caroli*; Rodentia). Among these animals, *C. horsfieldi*, as well as suncus and *Mus caroli*, contained NGF both in the submandibular and prostate glands. Therefore we investigated the sexual difference of NGF content in the submandibular gland of *C. horsfieldi* and studied the NGF localization in its submandibular and prostate glands compared with those of suncus.

Materials and Methods

C. horsfieldi, captured at Tainan (Taiwan) in February 1986 and 1987, and suncus, fed in our laboratory, were used. The animals were killed ether, and the submandibular and prostate glands were dissected out immediately. The glands were frozen with dry ice-acetone and kept at −70 °C. Thawed glands were homogenized in a glass homogenizer with 20 vol. of distilled water at 4 °C and centrifuged (3000 *g*, 2 h, 4°C). Centrifuge supernatants were stored at −70 °C and assayed for NGF activity and protein content within 4 weeks. Determination of NGF concentration was

performed following Saito *et al.*[5] Briefly, 8- to 10-d-old chick embryonic dorsal root ganglia were cultured with the homogenized supernatant for 24 h (Dulbecco's modified Eagle's medium, plasm clot method, 37 °C), and neurite outgrowth were scored from 0 to 8. The sample concentration inducing score 4, a dense halo of neural fibers, was defined as 1 biological unit (BU)/ml. Protein was assayed following Bradford.[6] For the study of NGF localization, adult male *Mus caroli* were fixed with cardiac perfusion of Bouin's solution. Submandibular and prostate glands were mounted in paraffin and 7 μm sections were made. An indirect immunohistochemical method was employed following Hazen-Martin and Simon.[7] Anti-mouse NGF antibody (rabbit, polyclonal, 75 μg/ml) and peroxidase-conjugated anti rabbit-IgG (goat, × 100, Jackson Immuno Research Lab.) were used as primary and secondary antibodies. The substrate of peroxidase was diaminobenzidine in the presence of hydrogen peroxide (20 mg/100 ml, 0.005% in Tris buffer, pH 7.6). Counter-staining was performed with hematoxylin. Mouse NGF was purified by the method of Bocchini and Angeletti,[8] and anti-mouse NGF antibody by the method of Stoeckel *et al.*[9]

Results

Typical NGF activities of the submandibular and prostate gland of *C. horsfieldi* are shown in Fig. 1. Chick embryonic dorsal root ganglion cultured for 24 h showed conspicuous neurite outgrowth, a dense halo of neural fibers, when the submandibular (Fig. 1A) and prostate (Fig. 1B) gland extracts were added to the culture medium (13.5 and 292 μg protein/ml respectively). Although adult male *C. horsfieldi* possessed NGF (116 BU/mg protein) in the submandibular gland, females (body weight 9 and 10 g) did not show NGF activity. In the submandibular glands of *C. horsfieldi* and suncus, NGFs were observed in the striated duct portion (Figs. 2A and 3A, respectively, arrows) but not in the secretory portion (Figs. 2A and 3A, respectively, arrowhead) in either species. In the prostate glands, NGFs were observed at the glandular epithelial cells (arrow) and also in the lumen (asterisk) both in *C. horsfieldi* and suncus (Figs. 2B and 3B, respectively).

Discussion

C. horsfieldi (Insectivora; Soricidae; Crocidura), as well as suncus (Insectivora; Soricidae; Suncus), had detectable levels of NGF activities in both the submandibular and prostate glands (Fig. 1). NGF content of the *C. horsfieldi* submandibular glands showed sexual difference, and the females did not possess NGF (see above). In suncus, females have only 10% of the NGF level of males.[10] Female mice also have less NGF (12.5%) than males in their submandibular glands.[11] Raynaud[12] has shown that submandibular gland of *C. horsfieldi*, like those of mice and suncus,[10,13] has male dominant sexual dimorphism in the

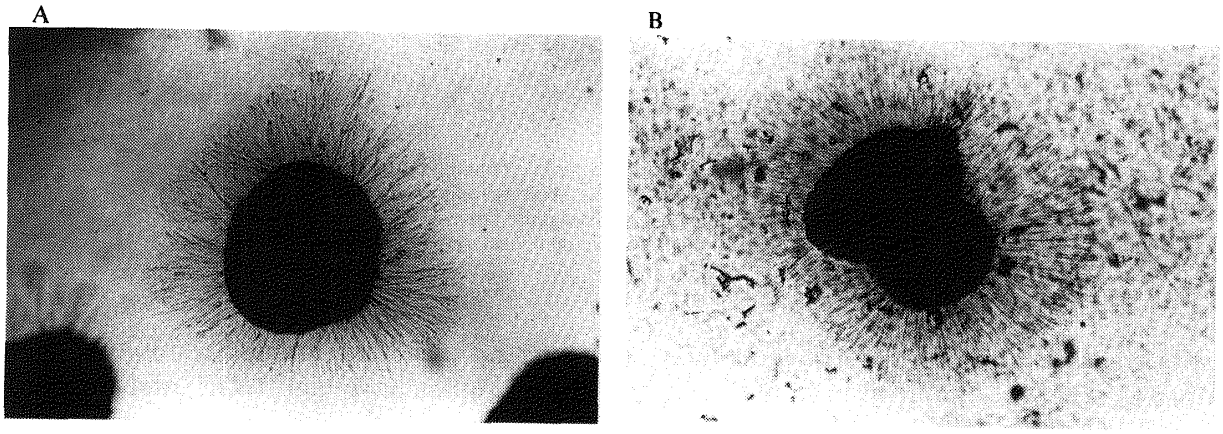

Fig. 1. Effect of *C. horsfieldi* Submandibular and Prostate Gland Extracts on the Neurite Outgrowth of the Embryonic Chick Dorsal Root Ganglion in Culture

Typical fiber outgrowth (a dense halo of neural fibers) was observed (A) at the submandibular extract concentration of 13.5 μg protein/ml, and (B) at the prostate gland extract concentration of 292 μg/ml.

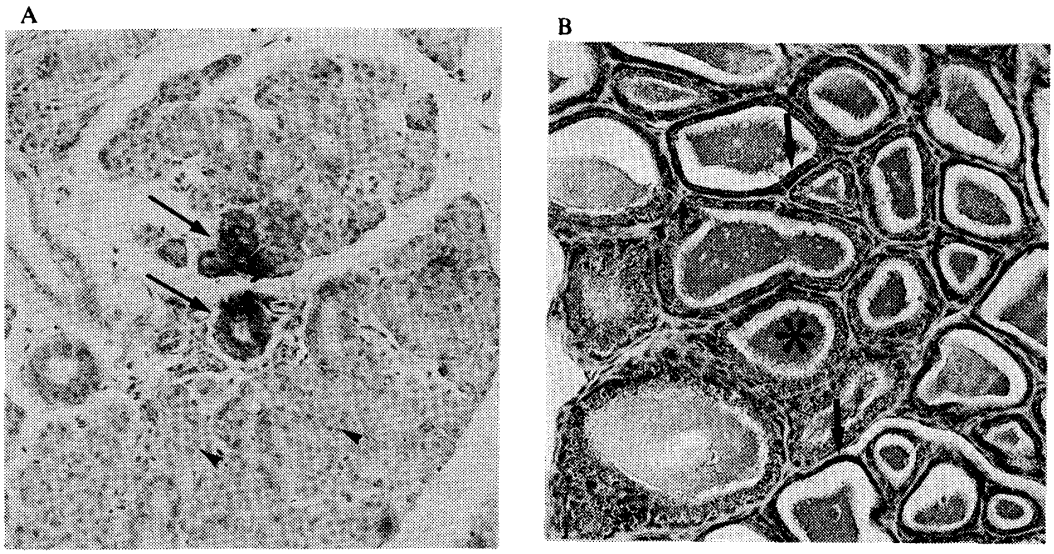

Fig. 2. Immunohistochemical Localizations of NGF in the Submandibular and Prostate Glands of *C. horsfieldi*

NGF localization was studied by utilizing an indirect immunohistochemical technique. A: Adult male submandibular gland. NGF localization was observed only in the striated duct portion (arrow), while the secretory portion was NGF-negative (arrowhead). B: Adult male prostate gland. NGF localized at the glandular epithelial cells (arrow) and in the lumen (asterisk).

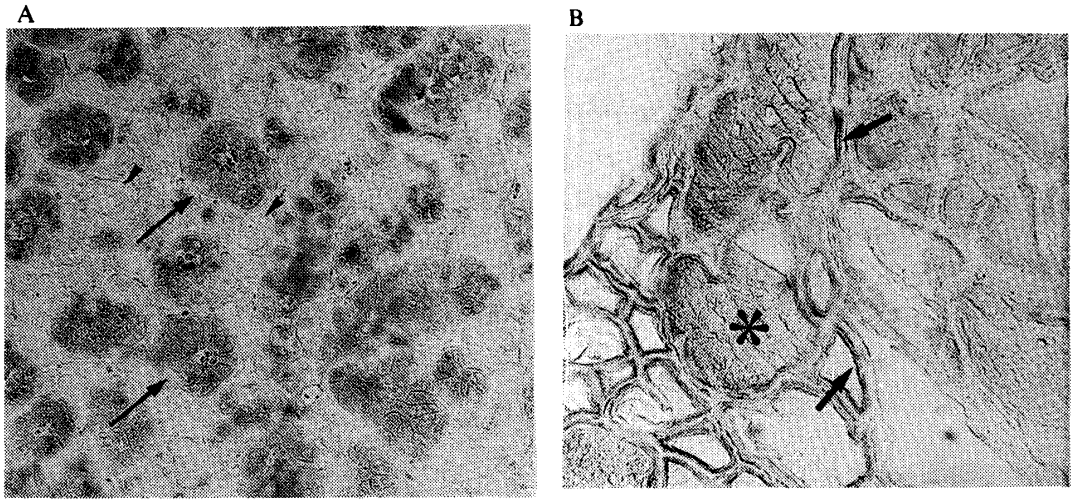

Fig. 3. Immunohistochemical Localizations of NGF in the Submandibular and Prostate Glands of *Suncus murinus*

NGF localization was studied by utilizing an indirect immunohistochemical technique. A: Adult male submandibular gland. NGF was observed only in the striated duct portion (arrow), while the secretory portion was NGF-negative (arrowhead). B: Adult male prostate gland. NGF was localized at the glandular epithelial cells (arrow) and in the lumen (asterisk).

striated duct portion. This phenomenon supports the idea that animals exhibiting male dominant sexual dimorphism in their submandibular glands have high NGF, and the NGF content of the gland is larger in males than in females.

In the submandibular glands of *C. horsfieldi* and suncus, NGFs were detected only in the striated portion (Figs. 2A and 3A, respectively). Although suncus has secretory granules in the secretory portion as well,[10] NGF could not be detected at that portion (Figs. 2A and 3A, respectively). Because mouse submandibular glands were shown to have the same NGF

localization,[14] there may be a close relationship between the above mentioned hypertrophy of the striated duct portion and the presence of NGF.

In the prostate glands, NGFs were detected in the glandular epithelial cells and also in the lumen of *C. horsfieldi* and suncus (Figs. 2B and 3B, respectively). This localization pattern is the same as those of guinea pig[15] and the field mouse *Mus caroli* (our unpublished observations). No linkage between the presence of NGF and morphological features has been found. However, this NGF localization pattern could be considered as generally occurring when NGF exists in the prostate gland. The possible reasons why NGF could be detected in the lumen may be as follows; first, the flow rate of the prostatic fluid is low, and second, there might be some unknown substance(s) which binds to NGF and prevents its efflux. The existence of the corpus amylacea, which was found in the suncus prostatic lumen[16] and in the rabbit prostate gland, might support the latter idea.

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