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Distribution of neurofilament-immunoreactive nerve fibers in human skin

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Summary. Neurofilament immunoreactive nerve fibers were demonstrated in human skin using indirect immunohistochemical technique with antibodies to neurofilament polypeptides. Neurofilament-positive fibers were seen as free nerve endings in the epidermis and in dermal papilla, in Meissner's corpuscles and as fibers crousing in the dermis. Strongly fluorescent nerve fibers were also seen around hair follicles, sweat gland ducts and sometimes in relation to blood vessels. From the distribution pattern it was concluded that predominantly sensory nerve fibers were labelled and that this technique may be used to study reinnervation of cutaneous sensory nerved following tramatic injuries and surgical procedures.

Introduction

Neurofilaments (NF), which are the neuronal cytoplasmic intermediate filaments, are built up of a triplet of polypeptides with molecular weights of approximately 70, 150 and 200 K (Hoffman and Lasek 1975; Schlaepfer 1977; see also Lazarides 1980). These polypeptides are chemically and immunologically distinct from the 50 K glial fibrillary acid protein and have been shown to comigrate in axoplasmic transport (Hoffman and Lasek 1975) and disappear in sciatic nerve undergoing Wallerian degeneration (Schlaepfer and Micko 1978). Most polyclonal NF-antisera, including the one used in the present study, seem to react with all three polypeptides in the NF-triplet (Dahl 1980, 1981; Dahl et al. 1981, 1982). In both the central and peripheral nervous system myelinated axons and a population of neuronal cell bodies are strongly immunoreactive (Björklund et al. 1984; Dahl and Bignami 1977; Dahl et al. 1981; Schlaepfer and Lynch 1977; Seiger et al. 1984; Shaw et al. 1981). However, not all neuronal cell bodies are stained by NF-immunohistochemistry (Dahl and Bignami 1977; Seiger et al. 1984; Shaw et al. 1981). It is not known whether both sensory and autonomic peripheral nerve fibers may be immunoreactive although it has recently been shown that the dense network of NF-positive fibers in the rat iris is of exclusively sensory origin (Seiger et al. 1984). In the present study the distribution of NF-like immunoreactivity in the human skin was investigated using indirect immunohistochemical technique.

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Material and methods

Blocks of human skin were taken with a 3 mm punch from the palmar aspect of distal phalanges or from the arm of healthy volounteers (the authors). The specimens were kept in a Ringer-sucrose solution, rapidly frozen, cut at 14 μm on a cryostat and processed for indirect immunohistochemistry according to Coons (1958). Briefly, the sections were incubated in a humid atmosphere at 4° C overnight with NF-antibodies raised in rabbits against degraded antigen from phosphate buffer extracts of autolyzed human spinal cord (Dahl and Bignami 1977). The NF-antisera was diluted 1/1000 in phosphate buffered saline (PBS). The second day the sections were rinsed in PBS, incubated with rhodamine-conjugated swine anti-rabbit antibodies (dilution 1:50, Dakopatts, Denmark) at room temperature for 60 min in darkness, rinsed in PBS, mounted in 90% glycerin in PBS and examined in a Zeiss fluorescence microscope. Control sections were incubated with preimmune serum or PBS.

Results

In human skin a dense pattern of NF-positive nerve fibers was observed (Fig. 1). The NF immunoreactive fibers were localized in the epidermis and dermal papilla as free nerve endings (Fig. 2A) and in Meissner's corpuscles (Fig. 2B). Several NF-positive nerve fibers were observed in the dermis (Fig. 1) in relation to blood vessels (Fig. 2C), sweat gland ducts (Fig. 2D) and in hairy skin in relation to hair follicles (Fig. 2E), whereas other fibers were seen crousing towards the dermal papilla and epidermis (Fig. 1). No immunoreactivity was observed in the control sections.

Discussion

The present study give strong evidence for NF-like immunoreactivity in a population of cutaneous nerve fibers. The NF-positive nerve fibers were localized in both the epidermis and dermal papilla as free nerve endings or in Meissner's corpuscles, and in the dermis suggesting mainly a sensory origin.

The NF-antiserum was first developed by Dahl and Bignami (1977) and have since then been used extensively for studies of neuronal development (Bignami et al. 1980; Raju et al. 1981; Raju and Dahl 1982), degeneration and regeneration (Chi et al. 1980; Dahl and Bignami 1978; Dahl et al. 1982) and normal neuronal structure (Björklund et al. 1984; Dahl and Bignami 1977; Seiger et al. 1984). The antisera used in the present study have been tested in immunoblotting experiments and was shown to recognize all three NF-

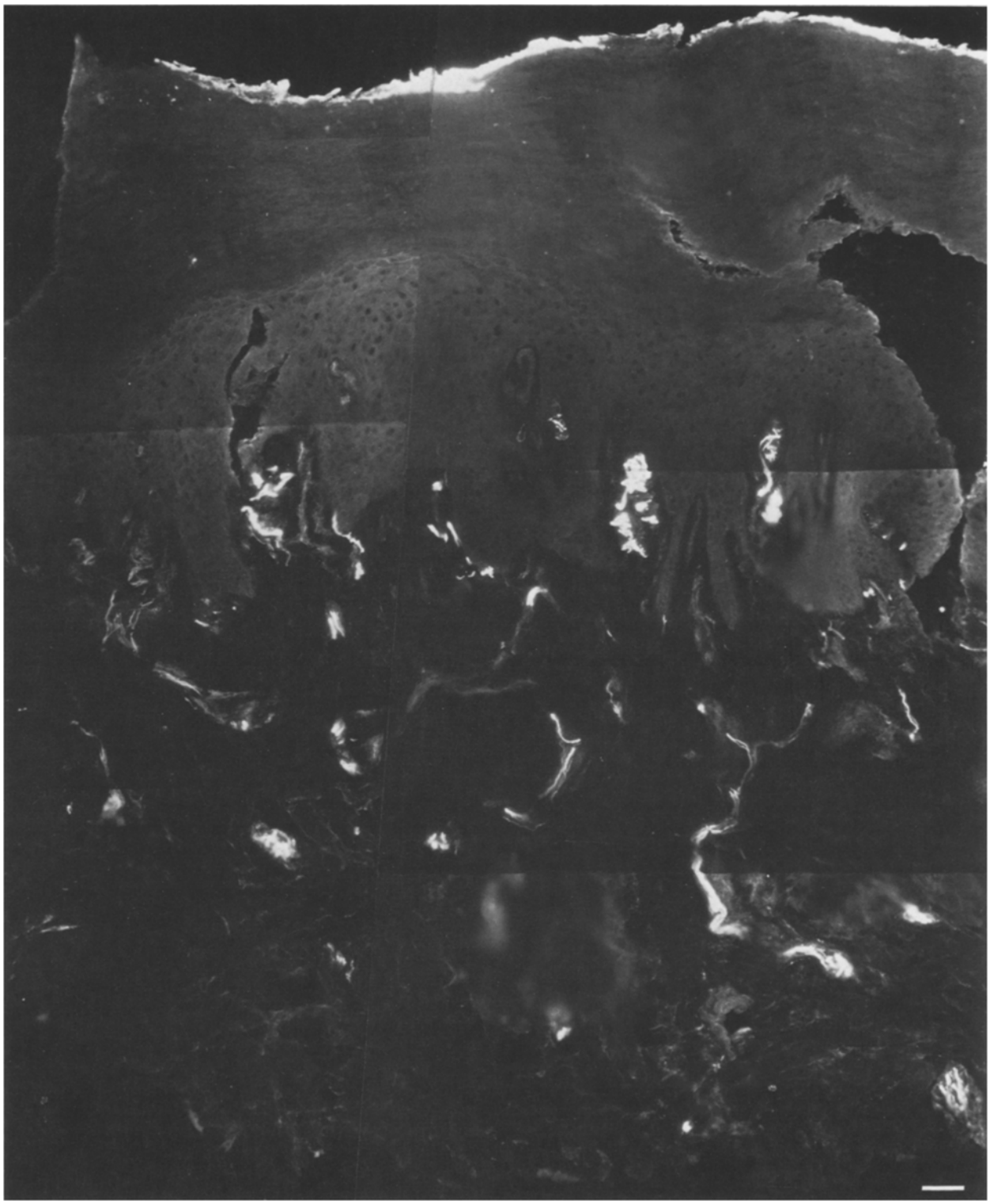

Fig. 1. Immunofluorescence micrograph of a cryostate section of human digital skin after incubation with NF-antiserum. Strongly fluorescent nerve fibers are observed in the dermal papillae as free nerve endings or in Meissner's corpuscles. NF-positive fiber bundles are also frequently seen in the dermis (*arrows*). Scale bar = 50μm

polypeptides (Dahl et al. 1982; Raju and Dahl 1982; Seiger et al. 1984).

The distribution and morphology of the NF-positive nerve fibers found in the present study resemble the distribution of myelinated and unmyelinated sensory fibers described in earlier light and electronmicroscopical studies (Cauna 1956, 1980; Miller et al. 1958). The finding of NF-positive fibers in Meissner's corpuscles is in line with the findings in a recent study of Iwanaga et al. (1982) where a comparison of S-100-protein-, neuronspecific enolase- and NF-like immunoreactivity was performed. In this study it was reported that the lamellar cells of the Meissner's

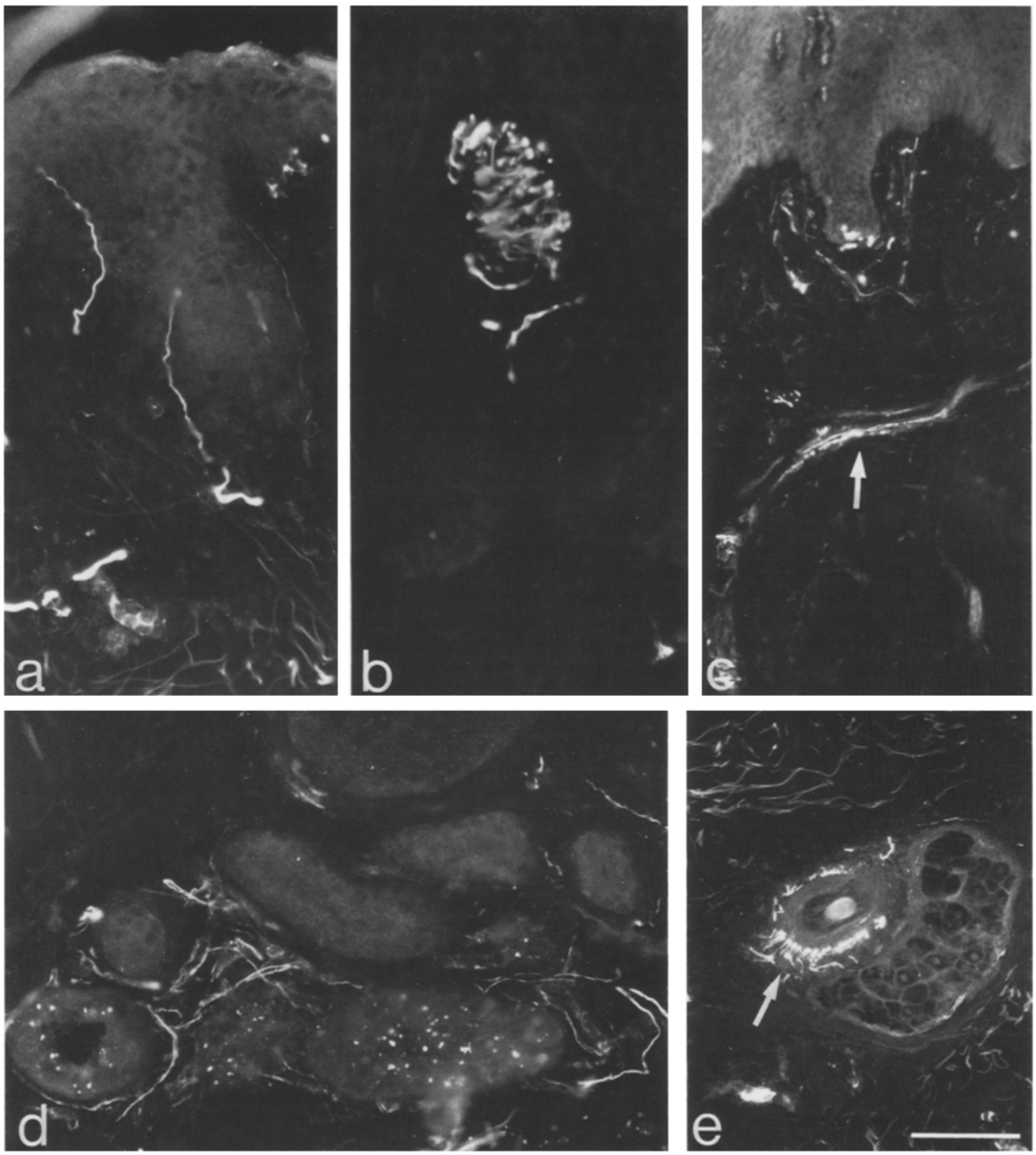

Fig. 2a–e. Immunofluorescence micrographs of cryostate sections of human digital skin (**a–c**) and human arm skin (**d–e**) after incubation with NF-antiserum. Immunoreactive nerve fibers are seen in the epidermis as free nerve endings (**a**), in dermal papillae in Meissner's corpuscles (**b**) and in the dermis around blood vessels (**c**), sweat gland ducts (**d**) and hair follicles (**e**). Scale bar = 50 μm

corpuscles were S-100-protein-positive, suggesting that these cells are derived from Schwann cells, and that nerve fibers were both NF- and neuronspecific enolase-positive.

In a recent study we have demonstrated substance P immunoreactive nerve fibers in human digital skin (Dalsgaard et al. 1983). The distribution of substance P-positive fibers was somewhat different from that of the NF-positive fibers described in this study; substance P-positive nerve fibers were often seen as free nerve endings in the dermal papilla and epidermis and only occasionally in Meissner's corpuscles whereas NF-positive nerve fibers was frequently observed both in Meissner's corpuscles and as free nerve endings in the dermal papilla and epidermis. A discrepancy between the distribution of NF- and substance P-positive sensory fibers was also observed in the rat iris (Seiger et al. 1984). Furthermore, in iris sensory nerve fibers, but not autonomic nerve fibers, were NF-positive indicating that NF-antibodies might selectively label afferent fibers.

The functional role or transmitter content of the NF immunoreactive sensory fibers in the digital skin is not yet known. Since NF-like immunorectivity was observed in free

nerve endings and in Meissner's corpuscles it is suggested that the NF-positive nerve fibers are involved in the mediation of several sensory modalities i.e. touch, heat and pain. In a study of the rat trigeminal ganglion Seiger et al. (1984) showed that about 80% of the cell bodies were NF immunoreactive, indicating that several sensory functions and neurotransmitters may be involved.

In conclusion. NF-immunoreactive nerve fibers were demonstrated in the human skin. The distribution of these fibers suggests a sensory origin, and that sensory fibers with different functions are NF-immunoreactive. The present technique may thus be suitable for selective studies of the sensory reinnervation in the skin, which has previously not been possible.

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References

Bignami A, Dahl D, Seiler MW (1980) Neurofilaments in the chick embryo during early development. I. Immunofluorescence study with antisera to neurofilament protein. Dev Neurosci 3:151–161

Björklund H, Dahl D, Seiger Å (1984) Neurofilament and glial fibrillary acidic protein related immunoreactivity in rodent enteric nervous system. Neuroscience (in press)

Cauna N (1956) Nerve supply and nerve endings in Meissners corpuscles. Am J Anat 99:315–350

Cauna N (1980) Fine morphological characteristics and microtophography of free nerve endings of the human digital skin. Anat Rec 198:643–656

Chi NH, Bignami A, Bich NT, Dahl D (1980) Autologous sciatic nerve grafts to the rat spinal cord: Immunofluorescence studies with neurofilament and gliofilament (GFA) antisera. Exp Neurol 68:568–580

Coons AH (1958) Fluorescent antibody methods. In J.F. Danielli (ed): General cytochemical methods. Academic Press, New York, pp 399–422

Dahl D (1980) Study on the immunological crossreactivity of neurofilament polypeptides in axonal preparations of bovine brain. FEBS Lett 111:152–156

Dahl D (1981) Isolation of neurofilament proteins and of immunologically active neurofilament degradation products from extract of brain, spinal cord and sciatic nerve. Biochim Biophys Acta 668:299–306

Dahl D, Bignami A (1977) Preparation of antisera to neurofilament protein from chicken brain and human sciatic nerve. J Comp Neurol 176:645–658

Dahl D, Bignami A (1978) Immunochemical cross-reactivity of normal neurofibrils and aluminium-induced neurofibrillary tangles. Immunofluorescence study with antineurofilament serum. Exp Neurol 58:74–80

Dahl D, Bignami A, Bich NT, Chi NH (1981) Immunohistochemical localization of the 150 K neurofilament protein in the rat and the rabbit. J Comp Neurol 195:659–666

Dahl D, Selkoe DJ, Pero RT, Bignami A (1982) Immunostaining of neurofibrillary tangles in Alzheimer's senile dementia with a neurofilament antiserum. J Neurosci 2:113–119

Dalsgaard C-J, Jonsson C-E, Hökfelt T, Cuello AC (1983) Localization of substance P-immunoreactive nerve fibers in the human digital skin. Experientia 39:1018–10120

Hoffman PN, Lasek RL (1975) The slow component of axonal transport. Identification of major structural polypeptides of the axon and their generality among mammalian neurons. J Cell Biol 66:351–366

Iwanaga T, Fujita T, Takahashi Y, Nakajima T (1982) Meissner's and pacinian corpuscles as studies by immunohistochemistry for S-100 protein, neuron-specific enolase and neurofilament protein. Neurosci Lett 31:117–121

Lazarides E (1980) Intermediate filaments as mechanical integrators of cellular space. Nature 283:249–256

Miller MR, Ralston HJ, Kasahara M (1958) The pattern of cutaneous innervation of the human hand. Am J Anat 102:183–218

Raju T, Bignami A, Dahl D (1981) In vivo and in vitro differentiation of neurons and astrocytes in the rat embryo. Dev Biol 85:344–357

Raju TR, Dahl D (1982) Immunofluorescence staining of cultured neurons: A comparative study using tetanus toxin and neurofilament antisera. Brain Res 248:196–200

Schlaepfer WW (1977) Immunological and ultrastructural studies of neurofilaments isolated from rat peripheral nerve. J Cell Biol 74:226–240

Schlaepfer WW, Lynch RG (1977) Immunofluorescence studies of neurofilaments in the rat and human peripheral and central nervous system. J Cell Biol 74:241–250

Schlaepfer WW, Micko S (1978) Chemical and structural changes of neurofilaments in transected rat sciatic nerve. J Cell Biol 78:369–378

Seiger Å, Dahl D, Ayer-Le Lievre C, Björklund H (1984) Appearance and distribution of neurofilament immunoreactivity in iris nerves. J Comp Neurol 223:457–470

Shaw G, Osborn M, Weber K (1981) An immunofluorescence microscopical study of the neurofilament triplet proteins, vimentin and glial fibrillary acidic protein within the adult rat brain. Eur J Cell Biol 26:68–82