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Light and Electron Microscopical Studies of the Substance P Innervation of the Dorsal Column Nuclei and the Lateral Cervical Nucleus in the Primate

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ABSTRACT

In 2 monkeys of the species Macaca fascicularis the three dorsal column nuclei and the lateral cervical nucleus have been investigated immunocytochemically with antiserum against Substance P.

The Substance P labeling was widely spread but rather sparse. It occurred in small structures of the size of boutons in the gracile, main cuneate and lateral cervical nucleus. The most intensive labeling of the gracile nucleus was found in the dorsal border of the nucleus and the lateral part of the caudal division. At the border between the spinal trigeminal nucleus and the triangular part of the cuneate nucleus Substance P labeling was also increased but mainly localized to the former nucleus. In the pars rotunda and the caudal part of the main cuneate nucleus there was a more intense labeling laterally especially at the obex level. In the lateral cervical nucleus Substance P positive structures seemed evenly spread and somewhat more numerous than in the gracile and main cuneate nucleus. Electron microscopy demonstrated Substance P positive boutons, which were fairly large and mostly in synaptic contact with dendrites.

The results from the different nuclei in the monkey were compared with the results of similar investigations in the cat. It is concluded that there are important species differences especially on the light microscopical level in the lateral cervical nucleus. Thus Substance P terminals are evenly spread over the nucleus in the monkey whereas in the cat those structures are concentrated to the ventromedial region.

INTRODUCTION

The nerve cells of dorsal column nuclei (DCN) and the lateral cervical nucleus (LCN) receive somatosensory information from the body and pass their axons to higher levels of the brain especially the ventrobasal thalamus. So far most studies of the DCN and LCN have been performed in rats and cats.

These studies have demonstrated the neuronal populations and their connections (For references see 2,5) and also some of the neurotransmitters and neuropeptides within the nuclei (1,3,4,6,10,12).

So far, however, no studies of transmitters and peptides in primates have been performed in any of the lower somatosensory relay nuclei to confirm that findings made in lower mammals also are valid in the primates and thus possibly in man. In the present study the Crab-eating monkey, Macaca fascicularis, has been investigated for the presence of Substance P a neuropeptide that earlier has been detected in DCN and LCN (3,6,12). Preliminary results have been published (7).

MATERIALS AND METHODS

Two adult monkeys of the species Macaca fascicularis were used. Under anaesthesia induced by sodium pentobarbital (40-60 mg/kg), the animals were perfused through the heart with 4.0% formaldehyde in 0.1 M phosphate buffer (38°C, pH 7.4) preceded by a rinse with 0.1 M phosphate buffer. After termination of the perfusion the brain and spinal cord were removed and kept in fresh fixative at 4°C.

Thereafter the lower brain stem and upper cervical spinal cord containing the DCN and the LCN were cut transversely on a Vibratome (Oxford Instruments) into sections 60 µm thick. The sections were preincubated in phosphate-buffered saline (PBS) with 0.2% Triton X-100 for one hour at 20°C before incubation in rabbit anti-Substance-P-antiserum (Immunonuclear, Stillwater, IL. USA) diluted 1:3000 for 3 days at 4°C. After rinsing in PBS, the sections were transferred to the secondary antibody solution (swine anti-rabbit 1:30 in PBS) and incubated for 60 min at room temperature under agitation; thereafter they were incubated in the peroxidase anti-peroxidase complex solution (1:120 in PBS) under the same conditions. The immunocomplexes were localized by incubating the sections for 3-6 min in a solution containing 75 mg of diaminobenzidine and 30 µl of 30% H_2O_2/100 ml of Tris-HCl buffer. The reaction was terminated by transferring the sections to a bath of Tris-HCl buffer. They were then postfixed for 20 minutes in 2% OsO_4 dissolved in cacodylate buffer, dehydrated in a graded series of ethanol and embedded in Epon between acetate foils.

After light microscopic investigation suitable Vibratome sections were mounted onto Epon blocks and resectioned for electron microscopy.

RESULTS

In the DCN light microscopy demonstrated a widely spread but rather sparse labeling of small structures with the size and shape of boutons and fibers

in the gracile and internal cuneate (Fig. 1A,B) but not in the external cuneate nucleus. The highest number of such structures in the gracile nucleus was found in the dorsal border of the nucleus and the lateral part of the caudal division. In the border between the spinal trigeminal nucleus and the triangular part of the cuneate nucleus the number of Substance P positive structures was increased but mainly localized to the former nucleus. In the pars rotunda and the caudal part of the main cuneate nucleus there was a more intense labeling laterally especially at the obex level.

In the LCN the substance P labeling seemed evenly spread both longitudinally and transversely and somewhat more intense than in the gracile and main cuneate nucleus (Fig. 2). In the electron microscope labeling of the DCN and LCN was demonstrated in mediumsized to large terminals (1-2 μm in diameter) mostly in synaptic contact with dendrites but especially in the LCN also with cell bodies. Due to the absence of glutaraldehyde from the fixative and the treatment with the detergent Triton the preservation of the tissue did not permit any conclusions regarding the form of the synaptic vesicles.

DISCUSSION

The distribution of Substance P in the DCN of the monkey seems to be different from that of the cat (12) at least on the light microscopical level. In the cat the Substance P activity was found in the ventral reticular areas of the internal cuneate nucleus where the muscle afferents terminate (9). In the monkey no such conclusions can be drawn at present although it is known that Substance P has an excitatory action on cuneate neurons (8).

Also in the monkey LCN the Substance P immunoreactivity is differently distributed than it is in the cat. Thus Substance P terminals in the monkey are evently spread over the transversal extent of the nucleus in contrast to the situation in the cat, where they are concentrated to the ventromedial half of the nucleus (3,6). It is interesting to note that the same species difference recently has been demonstrated for neuronal perikarya immunocytochemically positive for GABA (11). Thus the distribution both of an important inhibitory transmitter, GABA, and a neuropeptide, Substance P, underlines the special importance of the ventromedial part of the cat LCN, a part which in this animal is functionally segregated from the rest of the nucleus (for further discussion of this see e.g. 4).

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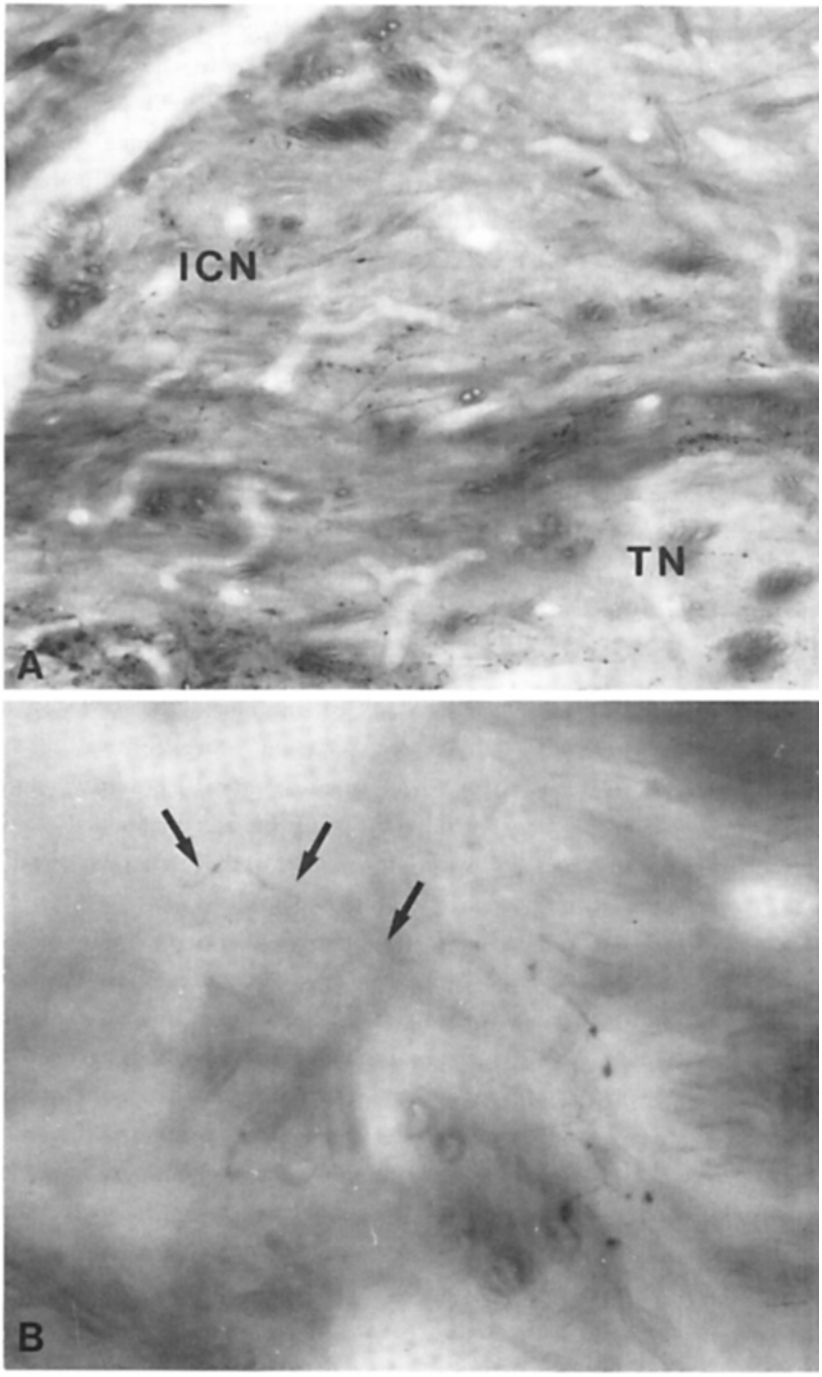

Fig. 1. Light micrographs of Substance P immunoreactivity of the DCN.

A. 210X Border zone between internal cuneate nucleus (ICN) and trigeminal nucleus (TN). Many of the labeled terminallike structures and fibers are situated within the former nucleus.

B. 640X Labeled fiber (arrows) and connected terminals from the middle of the gracile nucleus at the obex level.

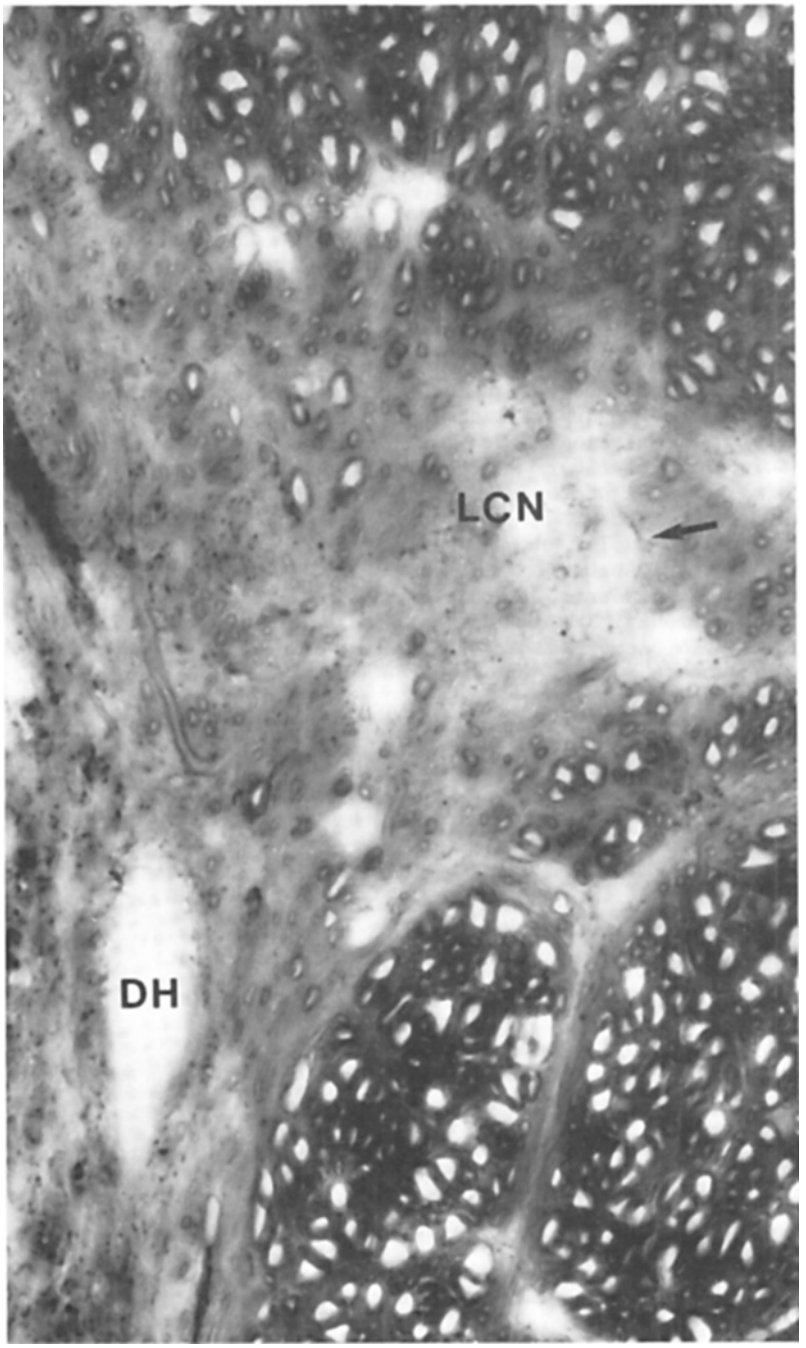

Fig. 2. Light micrograph of Substance P labeling of the LCN.

350X The LCN is here connected to the dorsal horn (DH). Note the numerous small labeled structures in the size of boutons in the LCN. Some seem to be located on a cell soma (arrow).

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