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Substance-P of neural cells in human trigeminal ganglion

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Expression of substance-P in human neurons of trigeminal ganglia has been investigated by immunohistochemistry and morphometry. These neurons constituted 12.8% to 32.6% of the total neuronal population in the trigeminal ganglia. Substance-P positive granulations were concentrated around the nucleus, distributed focally in neuroplasm or dispersed over the neuroplasm. Morphometric analysis has indicated the presence of three populations of SP-positive cells: small, medium-sized and large.

The results suggest a functional differentiation on the level of the first neurons of the afferent path of the stomatognatic system. Substance-P is likely to play a role in the transmission not only of nociceptive impulses but also of those involved in the mechano-functional stimulation of system activities.

key words: substance-P, human trigeminal ganglia, neurons, immunohistochemistry, morphometry

INTRODUCTION

Disturbances of the receptors of the stomatognatic system probably lead to changes in the antigenic character of endogenous proteins of the first trigeminal neurons in Gasserian ganglia. One of those proteins is substance-P /SP/ — neurotransmitter and/or modulator of nociceptive impulses [6,9–12,17,20,23]. Substance-P was discovered in 1931 by Van Euler and Gaddum [17,20] as a muscle stimulator of digestive system. This neuropeptide has been found in small neurons in different brain regions and in ganglion cells of dorsal horns of the spinal cord. The level of SP increases in diseases with pathological pain symptoms. Immunohistochemical analysis of SP in trigeminal ganglion has been carried out on both animal and human material [1–5,9,19,22]. Small and medium-sized SP-positive cells have been described.

Up till now, our knowledge of SP-positive neurons has not been thorough enough to give more information about their morphology in the human trigeminal ganglion. Therefore, in this study we decided to investigate the presence and localisation of SP in human Gasserian ganglia.

MATERIAL AND METHODS

15 human trigeminal ganglia (Gasserian) were dissected 6 to 12 hours after death during autopsy. The age of patients ranged from 38 to 86 years. Clinical and anatomopathological examinations excluded either neoplastic or other diseases with pathological pain concerning the nervous or stomatognatic system. In all the cases the condition of teeth was described as a partial loss or edentulous. The part of the ganglia containing the region between the maxillary and mandibular nerves of the fifth cranial nerve was isolated (Fig. 1) and immediately frozen at –70°C. Four μm thick sections were cut on Leica RM 2055 microtome. Immuno-

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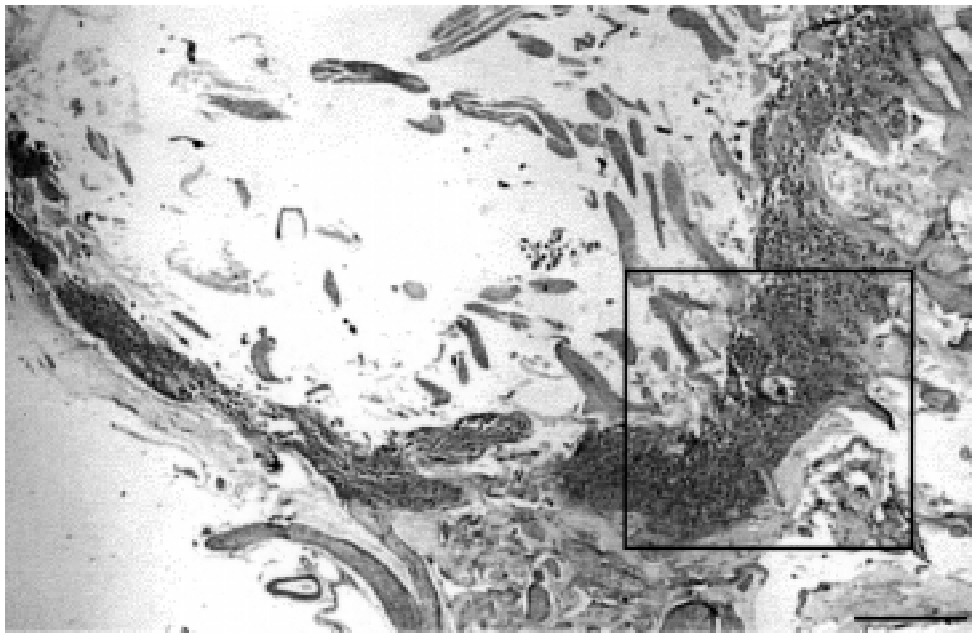

Figure 1. Human trigeminal ganglion — sampling probe (square) — scheme. Scale bar = 2.1 mm.

histochemical investigation of SP-positive neurons was performed by means of peroxidase method. The sections were incubated with antibody to substance-P (RPN 1572 anti-rabbit Ig, Amersham) diluted 1:10, at 4°C for 16 h. The second antibody was Peroxidase-Conjug pig anti rabbit Daco P-217, diluted 1:40, for 30 min. Meyer's method was used for haematoxylin counterstaining. For negative control the nonimmunologic rabbit serum was used. The sections were examined with a light microscope DMLS (Leica, Germany) co-operating with photocamera fixed to computer Pentium 75. SP-positive and SP-negative neuronal profiles, both with visible nuclei, were counted in 10 random test fields in each case of the investigated group. The percentage of SP-positive profiles of cells in relation to all neuronal profiles was calculated.

Detailed morphometric analysis of SP-positive neuronal profiles was carried out on the random test fields in five cases. Morphometric program Q500 MC (Leica, Germany) was used for measurements. Due to sectioning and sampling protocol we used a model based approach and measured the area of cross-sectional profiles of neurons. Altogether 120 profiles were analysed. According to their size, the cells were divided into the following groups: small cells — to 600 μm^2, medium-sized — from 601 to 1600 μm^2 and large — over 1600 μm^2. The results are presented in the graphs.

RESULTS

SP-positive neurons were observed in all investigated cases of human trigeminal ganglia (Fig. 2A,B). They constituted 12.8% to 32.6% of all cells (19.5% ± ± 5.8%) in the region between the maxillary and mandibular part of the trigeminal ganglion. In the neuroplasm the granulations of substance-P were either concentrated around the nucleus, distributed focally or dispersed over the neuroplasm.

A positive reaction was observed in cells different in size (Fig. 3).

The mean neuronal cross-sectional area was 935 μm^2 (± 390 μm^2). The majority of SP-positive neurons were of medium size. They constituted 75% of the population of immunoreactive neurons. The remaining cells were small (17%), or large (8%) (Fig. 4).

DISCUSSION

The occurrence of substance-P in first sensory neurons of afferent path of the trigeminal nerve localised in semilunary ganglia has been examined in three aspects. One of them is the ratio of the SP-positive cells to the total number of neurons in ganglia. Our results indicating such a varying percentage of SP-positive neurons in relation to all the cells in the region between the second and the third branches of the trigeminal ganglion may suggest different physiological characteristics of the investigated material. According to the literature in the human trigeminal

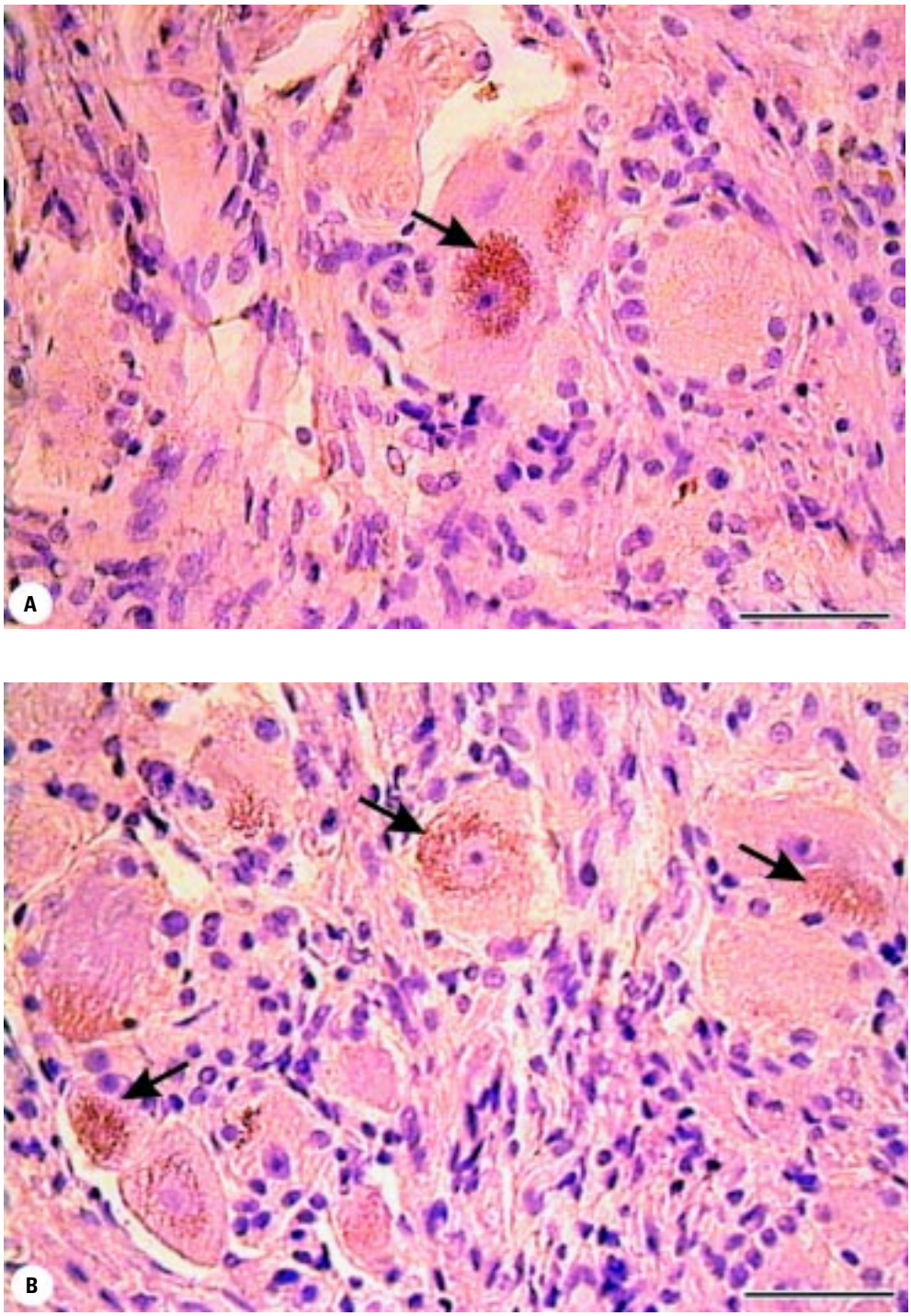

Figure 2. Human trigeminal ganglion — immunohistochemistry (peroxidase method). Arrows indicates SP — positive neurons. Scale bar = 0.05 mm.

ganglia the ratio of SP-positive cells to the total number of neurons calculated in newborns and adults was respectively 23.6 % and 16.7%, and the difference between them was significant [4]. The results of experiments on other species may serve only as comparative material. In monkeys the percentage of immunoreactive neurons was within a very close range in comparison to our findings in human material [21]. The cells constituted 16% to 32% of all the neurons. In rats the percentage of immunoreactive neurons was 38% [8,14]. Interestingly, a similar range (10% to 38%) was estimated for the distribution of

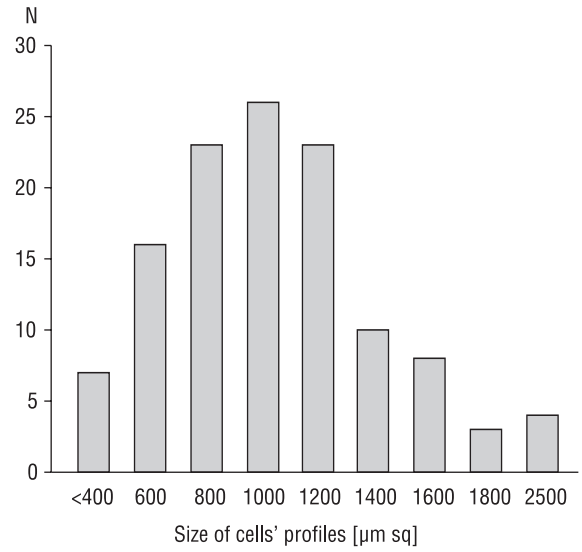

Figure 3. Distribution of the cross-sectional area of the SP-positive neurons.

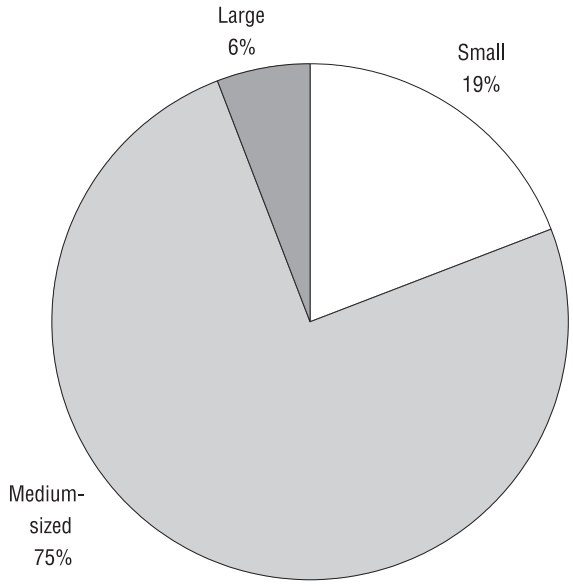

Figure 4. Percentage of the small, medium-sized and large SP-positive neurons.

SP-positive neurons in the dorsal ganglia [21,24], which may suggest the same role of the neurotransmitter in transmission of impulses on level of primary neurons.

There is no pattern of distribution of the immunoreactive product. Granulations of the SP have been observed in the whole neuroplasm, in a part of the cell or around the nucleus. Our results are in concordance with those from other experimental studies [1,11,14,15,18]. Different cytochemical characteristics of neurons in the trigeminal ganglion have been confirmed by results of experiments with double compound methods: axonal transport and immunohistochemistry [7,8]. The indirect immunofluorescence technique applied to human trigeminal ganglia identified two populations of neurons containing respectively high and low densities of immunoreactivity in the neuroplasm [4].

Till now a positive immunohistochemical SP-reaction has been observed mainly in small and medium-sized human and animal neurons in ganglia [3,9,10,13,14,16]. In contrast the present investigation has suggested that not only small and medium-sized cells are specific for SP-peptide. Heterogeneity in morphology SP-positive neuronal cells of the trigeminal ganglion may reflect differences in function of primary neurons in the afferent path of the trigeminal nerve or in the function of this neurotransmitter.

With our knowledge of substance-P, however, we are still not able to explain the function of SP neurons in transmission and/or modulation of nociceptive impulses. There are probably some additional factors that stimulate the endogenous protein. On the other hand, SP is likely to participate in the transmission of afferent impulses of other sensory modalities — not only nociceptive impulses, but also of those involved in mechano-functional stimulation of the system activities.

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