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De novo Expression of Neuropeptides in the Mesencephalic Trigeminal Nucleus of the Rat after Masseteric Nerve Injury

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In normal circumstances, galanin (GAL) and neuropeptide Y (NPY), two putative neuromodulators, are not expressed in the neurons of the mesencephalic trigeminal nucleus (MTN), a unique structure of (pseudo)unipolar sensory neurons in the CNS. Here we demonstrate GAL and NPY immunoreactivity in MTN neurons after an axotomy of *n. massetericus* in rats. Following survival periods of 7 and 14 days, the MTN neurons on the ipsilateral (axotomized) side display immunostaining, while on the contralateral side they are negative. It can be inferred that axonal injury initiates a cascade of intracellular events, leading to a *de novo* synthesis of neuroactive substances, such as GAL and NPY, which otherwise are not specific for the MTN neurons under normal conditions. The newly synthesized peptides possibly play a trophic role in the revival process or actively take part in re-establishing the MTN sensory modalities in the orofacial region.

Key words: axotomy, neuropeptides, mesencephalic trigeminal neurons, neurochemical plasticity, rat.

Introduction

Primary afferents in the trigeminal system have their cell bodies both in the trigeminal ganglion and the mesencephalic trigeminal nucleus (MTN) [6, 9]. The MTN is a distinctive structure because it is the only nucleus in the CNS predominantly consisting of (pseudo)unipolar neurons of a nerve crest origin, much like these in the peripheral sensory ganglia. The MTN perikarya are located in the midbrain and the rostral portion of the pons and send out their peripheral processes to muscle spindles of the jaw-closing muscles and extrinsic ocular muscles, and to mechanoreceptors in the periodontal ligament [8]. In normal circumstances the MTN utilizes in synaptic transmission a variety of neuroactive substances, although certain ones are expressed only under pathological conditions such as injury and pain. To date there exist no data on neuropeptide synthesis or expression in the MTN perikarya in health, notwithstanding the presence of fibre networks of varying density containing different neuropeptide substances amidst the neuronal bodies [6–8]. However, when subjected to injury of their peripheral processes,

the production of neuroactive substances and neuromodulators in MTN neurons, which normally are not a constituent of their milieu, is initiated [1, 4].

Thus, we set it as a goal of this study to test the MTN neurons in the rat for the expression of two neuropeptides, galanin (GAL) and neuropeptide Y (NPY) as a result of an acute trauma inflicted to the masseteric nerve unilaterally and to register chronologically the emergence and intensity of immunoreactivity, and its possible decrease.

Materials and Methods

Four adult rats underwent a unilateral transection of the *n. massetericus*. The untreated side served for a control. The animals were left to survive for 7 or 14 days. Then they were re-anesthetised and perfused with 4% paraformaldehyde. The brains were removed and the brainstem cut at the level of the MTN. After cryoprotection, the samples were cut on a freezing microtome at 20-30 μm. For immunostaining the sections were processed in accordance with the avidin-biotin-peroxidase complex (ABC) method [5]. Briefly, they were treated with 1.2% H_2O_2 in absolute methanol and preincubated in 3% normal goat serum in 0.01 M PBS containing 0.3% TritonX-100. Then they were incubated in primary polyclonal antibodies against GAL and NPY respectively, diluted 1:1000, in the preincubation medium for 48 h. The sections were consequently treated with biotinylated goat anti-rabbit IgG and the ABC complex. The peroxidase activity was visualized with 3,3′-diaminobenzidine. The sections were mounted on gelatin-coated glass slides and subsequently observed with a Zeiss AxioPlan 2 microscope.

Results

In the MTN of the animals subjected to unilateral transection of *n. massetericus*, prominent immunoreactivity (IR) was registered on the ipsilateral side of the axotomy 7 days after the intervention, while no IR was seen contralaterally (Figs. 1, 2). The IR was best expressed in the MTN neurons as well as in the surrounding nuclei, i.e. the locus ceruleus and the medial parabrachial nucleus. In the case of GAL, the IR was notably

Fig. 1. GAL-IR on the ipsilateral side of axotomy seven days after the intervention

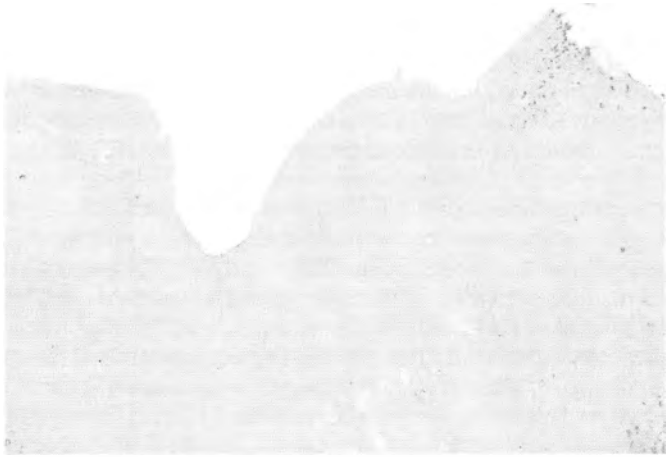

Fig. 2. NPY-IR seven days after axotomy. Although less pronounced, it is still well seen on the ipsilateral side of the intervention, while the contralateral remains negative

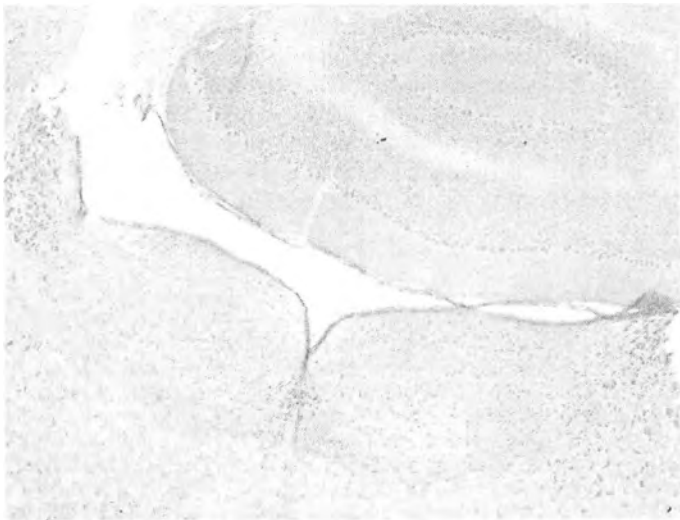

Fig. 3. Fourteen days after axotomy GAL-IR is still present, albeit in a weaker manner

expressed in the population of MTN neurons throughout the whole length of the nucleus, where they were visualized as dark-stained round-to-ovoid cell bodies against a negative background (Fig. 1). The GAL-IR was evenly dispersed throughout the perikarya. In the case of NPY the IR was clearly visible in the MTN neuronal population along the entire extent of the nucleus, although its intensity was somewhat weaker than that of the GAL-IR (Fig. 2).

We noted that 14 days following axotomy the overall IR, both of GAL and NPY, although much preserving the patterns as 7 days after the procedure, is in general notably weaker, which is a feature of a down-regulation in the expression of the two neuropeptides already commenced (Fig. 3).

Discussion

This study reveals the *de novo* synthesis and expression of two putative peptide neurotransmitters, GAL and NPY, in the MTN of the rat after peripheral transection of the *n. massetericus*. The obtained results are in agreement with the already published data by other authors, as well as earlier studies of ours [1, 2], confirming the induction of synthesis and upregulation of neuropeptides in the MTN neurons of the rat [1] and cat [6] following injury of their peripheral axons. For the first time we note that while there is a pronounced neuropeptide expression 7 days after axotomy, the intensity of the reaction diminishes two weeks following the intervention, which may be regarded as a sign of an already commenced down-regulation. The fact that MTN neurons react to peripheral axotomy by a *de novo* synthesis of neuropeptides such as GAL and NPY leads to the notion that these substances may play some central role in the trophic responses of neurons to the altered environmental cues. In this respect the study is in conformity with other sources reporting on the significant changes in the neurochemical content of primary sensory neurons following peripheral axotomy [1, 4, 9]. We support the view of these authors that obviously nerve damage causes the surviving neurons to shift their functional activities from normal maintenance and neurotransmission away to sustaining survival and regeneration. In our opinion, the newly synthesized neuropeptides, GAL and NPY, possibly play a supportive role as neurotrophic factors in the course of the adaptive processes that initiate and develop in response to injury. Thus they may protect the peripherally axotomized MTN neurons in their pathway back to re-establishing the usual functional modalities. Very recent data *in vitro* confirm that the MTN, similar to certain hypothalamic nuclei, possibly has a determinative role in setting the circadian rhythms and serves in regulating daily feeding behavior [3]. It may be speculated that the *de novo* expression of neuropeptides is one way of adaptation to a newly geared biological clock in accordance with the changed environmental conditions and that it assists in re-establishing the MTN sensory modalities in the orofacial region.

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