Mamillary Complex of Hypothalamus and Efferent Functions of Capsaicin-Sensitive Neurons

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Electrocoagulation in the region of mamillary complex of the hypothalamus induced disturbances in vascular permeability in the rat limb skin. This effect was prevented by pretreatment of the animals with the neurotoxin capsaicin, which indicates participation in the effect of the peptidergic capsaicin-sensitive neurons. The data are discussed in relation to possible mechanisms of central modulation of efferent function of the primary sensory neurons.

Key Words: *capsaicin; vascular permeability; neuropeptides; mamillary complex*

The peptidergic capsaicin-sensitive neurons (CSN), whose somata are located in the spinal and trigeminal ganglia, play an important role in mediating the early stages of neurogenic inflammation [2-4]. These neurons have double (afferent-efferent) functions: on the one hand, in response to peripheral stimulation they convey the nociceptive impulses to central nerve system by releasing substance P and calcitonin gene related peptide from the central termination in the spinal cord and metencephalon; on the other hand, they release the same neuropeptides from directly stimulated peripheral termination, which results in disturbance of microvessel permeability in the innervated tissues [3]. The efferent functions of CSNs depend not only on the intensity of peripheral stimulation, but also on morphofunctional condition of the sensory ganglia where the somata of these neurons are located [3,4].

Previously, we showed that neurogenic processes develop in peripheral tissues after destruction of cerebral nuclei in which the central terminations of the primary CSNs are located [6]. The appearance of neurogenic disturbances against the background deficiency of the afferent traffic to the prosencephalon structures, to thalamic and hypothalamic nuclei in particular, indicate modulation of CSN efferent functions by central mechanisms. In this study we chose the hypothalamic structures with due account of the influence of its nuclei on functional activity of vegetative centers of the spinal cord and metencephalon. It is supposed that the descending influences of hypothalamic and other structures of the prosencephalon on these centers are mediated mainly via hypothalamic mamillary complex due to its neuroanatomic location and the character of neural connections [10].

Our aim was to study how the destruction of hypothalamic mamillary nuclei affects cutaneous microvessel permeability in the limbs and assess the dependence of this influence on the functional state of primary CSNs. Therefore, some experiments were performed against the background of the neurotoxin capsaicin, which induced depletion of vasoactive neuropeptides from this group of neurons [3,4].

MATERIALS AND METHODS

Experiments were carried out under Nembutal anesthesia (40 mg/kg intraperitoneally) on male Wistar rats weighing 150-250 g. Stereotaxic right-hand electrocoagulation of the mamillary complex was performed in accordance with coordinates A=3 mm, L=0.5 mm, and H=10 mm [7]. Electrocoagulation was

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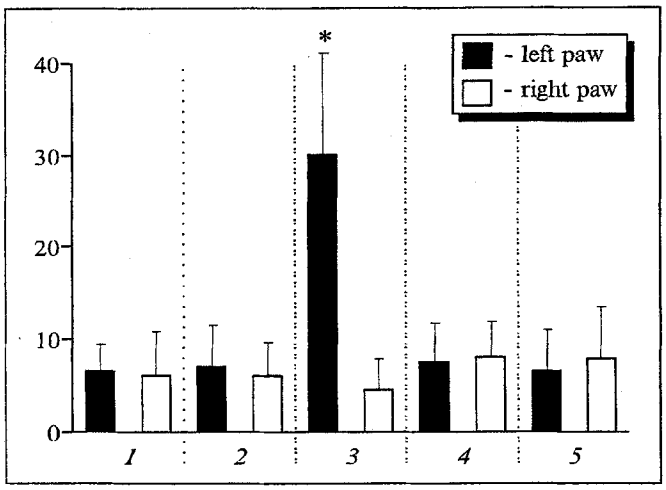

Fig. 1. Concentration of Evans Blue in the skin of thoracic limbs (ng/mg tissue) in different experimental groups. *1*) control (intact animals, which received Evans blue 1 h before killing); 2) sham operated animals (electrode insertion into the right part of the mamillary complex without electrocoagulation); 3) the right-hand electrocoagulation of the mamillary complex; *4*) animals treated with capsaicin a week prior killing; 5) right-hand electrocoagulation of the mamillary complex against the background of capsaicin. *$p<0.05$ in comparison with the control group.

done with direct current (5 mA during 30 sec). Unsuccessful operations were revealed morphologically. In sham operated rats, electrode was inserted into the right dorsal portion of the mamillary complex without electrocoagulation.

During four days, the animals of an individual group were injected subcutaneously with 1% capsaicin (Serva) in doses of 25, 25, 50, and 100 mg/kg [6]. Seven days after the last injection, the rats were subdivided into two subgroups. In one of these subgroups, the right dorsal portion of the mamillary complex was coagulated.

At the end of experiments, 1 h before killing the animals, Evans Blue was administered intravenously (50 mg/kg). The rats were decapitated, the portions of skin of the thoracic limbs were cut out and weighted. The material of each paw was placed in 3 ml formamide for 3 days at room temperature [9]. Optic density of the extracts was measured in an SF-46 spectrophotometer at 620 nm. The data were related to the tissue mass. The results were statistically analyzed using Student's *t* test. Intact animals injected with Evans Blue 1 h prior decapitation were taken as control.

RESULTS

Morphological studies showed that electrolytic destruction affected predominantly *n. mamillaris lateralis* and lateral part of *n. mamillaris medialis*. In addition, in different animals the coagulation zone spread to *fasciculus mamillo-tegmentalis*, *fasciculus mamillo-thalamicus*, and *n. premamillaris ventralis*.

In rats with right-hand electrocoagulation in the mamillary complex, a significant increase was observed in vascular permeability of the skin of the left thoracic limb compared with the right thoracic limb (Fig. 1). This increase was a consequence of nuclear destruction, and did not result from the procedure of electrode insertion into the studied structures, because the recorded values in the sham operated animals did not differ from the control (Fig. 1).

Opposite data were obtained in experiments with destruction in the region of mamillary complex one week after administration of neurotoxic dose of capsaicin, i.e., against the background of a profound decrease in the content of substance P and some other vasoactive neuropeptides in the peripheral nerve termination of spinal ganglionic CSNs [6]. Under these conditions, destruction of hypothalamic nuclei was not accompanied by changes in the permeability of cutaneous microvessels (Fig. 1). It should be noted that prior the operation accumulation of the dye in both thoracic limbs of the capsaicin-treated rats did not significantly differ from that of the control rats (Fig. 1).

Thus, electrocoagulation in the region of mamillary complex results in disturbance of vascular permeability in the peripheral tissues. The fact that this disturbance is prevented by pretreatment of the animals with capsaicin, indicates its participation in the effects produced by peptidergic CSNs. Electrocoagulation in the region of mamillary complex may enhance the release of neuropeptides from peripheral terminations of these neurons. The mamillary complex is known to incorporate the descending hypothalamic pathways participating in vasomotor regulation, which affects the activity of sympathetic and parasympathetic centers [10]. It seems probable that these pathways mediate the inhibitory influences on the spontaneous release of neuropeptides from peripheral terminations of CSNs. Elimination of this inhibitory influences by electrocoagulation in the region of mamillary complex results in enhancement of neuropeptide release from the periphery, which in its turn leads to an increase in vascular permeability. At present it is impossible to explain the mechanism of the central influences on the activity of peripheral peptidergic termination. They could be mediated by the receptors located directly on these termination. It has been shown that peripheral termination of sensory neurons have α_2-adrenoreceptors which can inhibit the release of neuropeptides from them [1,8].

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NADPH-Diaphorase in the Lungs of Rats with Experimental Bronchial Asthma

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Distribution and activity of NADPH-diaphorase colocalized with NO-synthetase are studied in bronchial epithelium, pneumocytes, and alveolar macrophages of rats with experimental bronchial asthma. Increased activity of NO-synthetase in these structures indicates that nitric oxide is involved in allergic inflammation.

Key Words: *asthma; nitric oxide; NADPH-diaphorase; macrophages; pneumocytes*

Recent studies show that nitric oxide (NO) is formed in the respiratory system [5,9,15]. Special attention has been focused on the role of NO in pulmonary pathologies, including bronchial asthma (BA) [3,6, 13]. There is evidence that the concentration of NO in the air exhaled by patients with BA is increased [3,13]; however, the role of NO in BA is not fully understood.

Our objective was to identify and quantitate NADPH-diaphorase, an enzyme participating in the synthesis of NO, in bronchial epithelium, pneumocytes, and lung macrophages of intact rats and rats with experimental BA.

MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 180-200 g. The animals were maintained under standard vivarium conditions. Experimental BA was produced as described [4] to ensure changes in the bronchopulmonary system typical of this disease [14]. The rats were sensitized for 2 days by subcutaneous infection of 10 μg ovalbumin dissolved in 0.5 ml of solution containing 100 mg $Al(OH)_3$. Three weeks after sensitization, the rats inhaled the resolving dose of ovalbumin in a concentration of 0.8 ml/min and were decapitated at the peak of bronchospasm. Intact rats served as the control. Morphological examination of the lungs of sensitized rats revealed changes typical of BA: deformation of bronchial wall and narrowing of the bronchi, hyperplasia of goblet cells, focal desquamation of the epithelium, and moderate hypertrophy of smooth muscles.

NADPH-diaphorase was identified by the method [12]. Pieces of lungs (1×0.5 cm) were fixed for 2 h at 4°C in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), which preserves the activity only of NADPH-diaphorase. The pieces were then washed at the same temperature in 15% sucrose. Cryostat sections (10 μ) were mounted on glass slides and incubated for 60 min at 37°C in a medium containing 50 mM Tris-HCl (pH 8.0), 1 mM NADPH (Sigma), 0.5 mM Nitro Blue Tetrazolium (Sigma),

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