Role of Capsaicin-Sensitive Neurons in the Regulation of Structural Organization of the Thymus

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Anatomical organization of the thymus was studied in adult Wistar rats after injection of a neurotoxic dose of capsaicin. The reaction of the lymphoid parenchyma attested to depletion of structural reserves and triggering of autoimmunization mechanisms and was associated with accumulation thymocytes with morphological signs of apoptosis, Hassal's bodies, macrophages, plasmacytes, and eosinophils in the subcapsular zone. Through neurokinin and vanilloid receptors on the sensory terminals and lymphocytes, capsaicin modulated the level of neuropeptides, which seemed to be involved in the regulation of autoimmune mechanisms.

Key Words: *thymus; capsaicin-sensitive neurons; neuropeptides; thymocyte apoptosis; neurokinin and vanilloid receptors*

Recently the thymus is defined as a neuroimmunoendocrine modulus. The role of the CNS as the most important factor of the thymus morphogenesis is intensely studied. However, the role of the afferent component of CNS (primary sensory neurons) in the regulation of anatomic organization of lymphoid organs is unclear. This is a new aspect in the studies of mechanisms regulating visceral functions and neuroimmune interactions. Dendritic terminals of primary afferent neurons of C2-C4 spinal ganglia realizing transmitter neuropeptides innervate simultaneously the dorsal horns of the spinal cord, the thymus, and subcutaneous connective tissue on the back [3]. High immunoreactivity to neuropeptides selectively modulated by capsaicin [6] was noted in the zone of their peripheral processes location. Neonatal injection of capsaicin modulates the immune response of blood lymphocytes [14], reduces cytotoxic activity of natural killer cells, and disturbs some stages of thymocyte differentiation [13]. However, this treatment is associated with degeneration of the sensory terminals, which can be compensated by activation of adrenergic and cholinergic systems. Subcutaneous injection of a neurotoxic dose of the drug to adult animals led to temporary blockade of neuropeptides of capsaicin-sensitive neurons (CSN) at the site of injection and in the thymus receiving direct projections of these neurons by the axon reflex mechanism. Capsaicin effect can be mediated through vanilloid receptors on sensory terminals and lymphocytes [8]. Pharmacological blockade of these receptors and intensive thermal and photoexposure decrease the level of neuropeptides in peripheral tissues [12]. Immunohistochemical studies showed that injection of a neurotoxic dose of capsaicin led to intensive release of neuropeptides from CSN terminals and subsequent (after 2 weeks) deficiency and recovery (after 3-4 weeks) of their content at the periphery [6]. With these data in mind, we analyzed the restructuring of the thymus after injection of the drug.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats (160-180 g) from Stolbovaya Breeding Center, kept under standard vivarium conditions. Capsaicin (Sigma, 1% solution in solvent: 10% ethanol, 10% Twin-80, 80% normal saline) was injected subcutane-

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ously under ether narcosis for 2 days at 12-h interval, in order to remove the sensitization effect, in doses of 25, 25, 50, and 50 mg/kg (total neurotoxic dose 150 mg/kg).

For light microscopy whole thymus or its part was fixed (within first few minutes after decapitation of animals under ether narcosis) in 10% neutral formalin or Telesnitskii fixative and embedded into paraffin. The sections were stained with hematoxylin and eosin and with Azur B-eosin Y after Nocht—Maximov, and by the method of Kramer for detecting mast cells. The absolute volume of structural functional zones in the thymus was evaluated in every 15th section (5 μ). Material for electron microscopy was fixed in 2.5% glutaraldehyde, postfixed in 2% osmic acid after Confield, and embedded into araldite and epon. The sections were made on an LKB-4800 ultramicrotome, contrasted with lead nitrate and uranyl acetate, and examined under a JEM-5-Y electron microscope. Semithin sections were stained with toluidine blue by the method of Unna. Counts of lymphocyte forms, reticular and plasma cells, phagocytes, cells with mitotic figures, and destructively changed cells were evaluated by the morphometrical method. Body weigh, thymus and kidney weights were measured. The thickness of the cortex and diameter of the medullary matter were evaluated by MOB-1 screw ocular micrometer. The significance of differences was evaluated using Student's *t* test.

RESULTS

After 2 weeks of capsaicin treatment experimental animals lost 12% body weight, morphophysiological index of their thymuses decreased 1.6-fold, corticomedullary index decreased 2.5 times in comparison with the control (corticomedullary index 3.2 in the control, 1.3 in experimental group), specific area of the cortical matter decreased by 26%, of the medulla increased by 1.8 times (Fig. 1). Morphometry of serial sections showed frequent inversion of the medullary and cortical matter in experimental group and a decrease in the absolute volume of the thymus (75.6 mm^3 in control, 52.8 mm^3 in experiment; $p<0.05$). This was paralleled by significant decreases in the volumes of the cortex (51.8 mm^3 in the control and 35.8 mm^3 in the experiment) and of the medulla (17.2 mm^3 in the control and 12.7 mm^3 in the experiment); connective tissue stroma tended to decrease (Fig. 2).

The fibers of primary afferent neurons abundantly innervate the subcapsular zone of the thymus and less so the intercapsular septae and parenchyma of the organ, where free processes lie between mast cells and $OX8^+$ thymocytes [3]. Total count of cells, counts of lymphocytes (blasts, medium-sized, minor), cells with mitotic figures and pyknotically-modified nuclei (apoptotic), plasmacytes, eosinophilic granulocytes, macrophages, mast cells, and epitheliocytes were evaluated per unit of subcapsular zone section (8800 μ^2) (Table 1). Total cell count in experimental rats decreased by 27% mainly at the expense of the decrease in lymphoid parenchyma (by 37%), the number of minor lymphocytes decreased by 28% and the count of apoptotic ones increased 7-fold (Table 1). Lymphoid cells can react to neuropeptide release through NK receptors [6] or to capsaicin through vanilloid receptors [8]. After neonatal capsaicin treatment the counts of $CD4^+$, $CD25^+$ lymphocytes increased in the peripheral blood of rats, this effect can be prevented by injections of SR 140333 [14]. Experimental rats exhibited a trend to an increase in the count of medium-sized (mature) lymphocytes, presumably, because of deceleration of their migration towards the site of evacuation from the

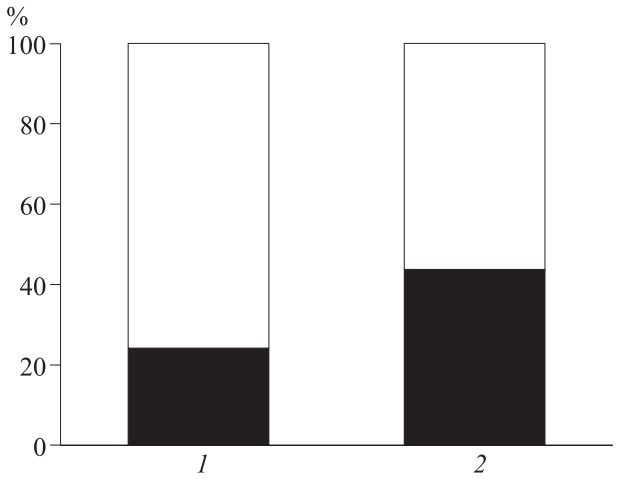

Fig. 1. Relative volume of the cortex (light fragments of bars) and medulla (dark fragments) of the thymus in control rats (*1*) and after 2 weeks of capsaicin treatment (150 mg/kg; *2*).

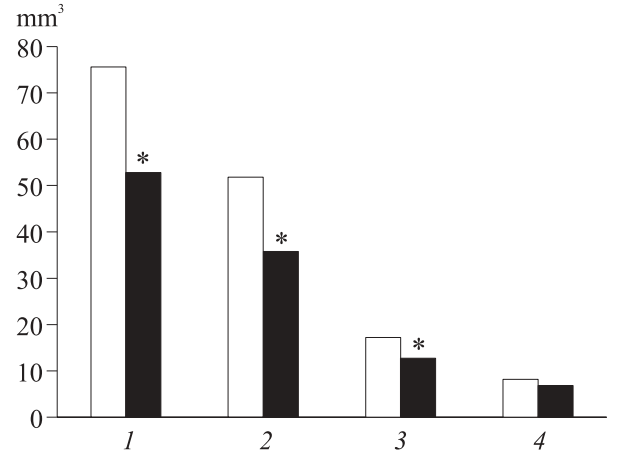

Fig. 2. Absolute volume of the thymus and its structural functional zones in Wistar rats of control (light bars) and experimental groups (dark bars). *1*) total volume; *2*) cortex; *3*) medulla; *4*) connective tissue stroma.

gland and decrease in the content of blast forms, indicating impaired lymphopoietic function. These effects can be due to neuropeptides, regulating the functions of E2/CD99 ($CD4^+$ and $CD8^+$ lymphocyte homotypical adhesion inductor) by stimulating lymphocyte adhesion to vascular cells and activate the release of mast cell transmitters heparin and histamine, inhibiting lymphocyte migration towards the site of antigen activity, and suppress blastogenesis [9].

Electronograms of experimental rats showed lymphocytes with morphological signs of apoptosis (chromatin condensation, formation of osmiophilic accumulations near the nuclear membrane, dilatation of endoplasmic reticulum, decreased size; Table 1) 7-fold more often than in the control. Delymphotization of the subcapsular zone of the thymus was associated with the appearance of numerous Hassal's bodies (sites of utilization of apoptotic thymocyte). Normally they are rare and are located in the medulla. Lymphocyte apoptosis can be stimulated by external, endogenous triggers and physiological factors in non-physiological concentrations and combinations. Neuropeptide imbalance stimulating expression of CD95 antigen (Fas or APO-1) on $CD4^+$, $CD8^+$, B-lymphocytes, granulocytes, monocytes, and natural killers can be such a factor. Impairment of autoimmune selection during attenuation of nervous regulation was previously hypothesized [10]; substance P was then regarded as a physiological antagonist of lymphocyte apoptosis [4]. Presumably, neuropeptide regulation of thymocyte apoptosis is realized through a cascade of caspases in the thymus, as intense thermal stimulation of sensory endings limits stress-induced activation of this complex [1].

In parallel with enhanced death of lymphoid cells in the subcapsular zone of the thymus in experimental rats, the number of macrophages with lipid incorporations and lymphocyte fragments increased 5-fold. Normally macrophages with morphologic signs of intense phagocytic activity, similarly as apoptotic thymocytes, are located in the medulla. The number of plasmacytes of different degree of maturation (including Mott cells) and eosinophils increased in the perivascular zones under the epithelium and at the corticomedullary interface of experimental rats attesting to activation of autoimmune processes [2]. Capsaicin induced a 3-fold increase in the number of thymic labrocytes (Table 1). Chains of 5-10 degranulating extralymphoid mast cells migrated into the thymic parenchyma, where they often released granules towards thymocytes (Fig. 3). Serotonin released by labrocytes increases vascular permeability modulating (by the feedback mechanism) vasodilatory activity of substance P; histamine (through H_3 receptors) provides triggering of autoimmune mechanisms [7]; gelatinase and fibronectin promote migration of labrocytes into deep regions of the parenchyma [9]. Non-degranulating mast cells with morphological signs of increased secretory activity accumulated near arterioles and postcapillary venules of the interlobular septae of the thymus. The mechanism of granule deposition in these mast cells is not quite clear. Presumably, substance P released under the effect of capsaicin blocks histamine H_3 autoreceptors on their cell membrane. Intrathymic labrocytes differ from the extralymphoid ones by the conditions of differentiation, their lipid structures increasing the production of immune factors in the absence of degranulation (Fig. 3). One more probable cause of this

TABLE 1. Cellular Composition of Subcapsular Zone of the Thymus in Control Wistar Rats and after 2 Weeks of Capsaicin Treatment in a Total Dose of 150 mg/kg (Absolute Count of Cells per Standard Area of 8800 μ^2; Percent of Total Cell Count; *M*±*m*)

Cells	Control	Capsaicin
Lymphocytes (total)	221.6±6.3 (93%)	140.3±3.8* (80.8%)
Blasts	40.30±4.27 (16.8%)	36.1±2.4 (20.8%)
Minor lymphocytes	145.7±2.7 (61.1%)	58.2±1.5* (33.5%)
Medium-sized lymphocytes	32.1±3.4 (13.5%)	36.9±2.6 (21.26%)
Lymphocytes with mitotic figures	2.40±0.44 (1%)	1.80±0.15 (1%)
Apoptotic lymphocytes	1.10±0.41 (0.5%)	7.2±1.7* (4.1%)
Macrophages	1.30±0.27 (0.5%)	6.5±1.1* (3.7%)
Epitheliocytes	14.10±2.75 (5.9%)	11.5±1.1 (6.6%)
Plasmacytes	1.20±0.17 (0.5%)	5.1±1.3* (2.9%)
Eosinophils	0.70±0.27 (0.3%)	7.1±1.1* (4.1%)
Mast cells	0	3.30±0.52* (1.9%)
Sum of all cells	238.7±4.4	173.6±4.6*

Note. *$p<0.01$ compared to the control.

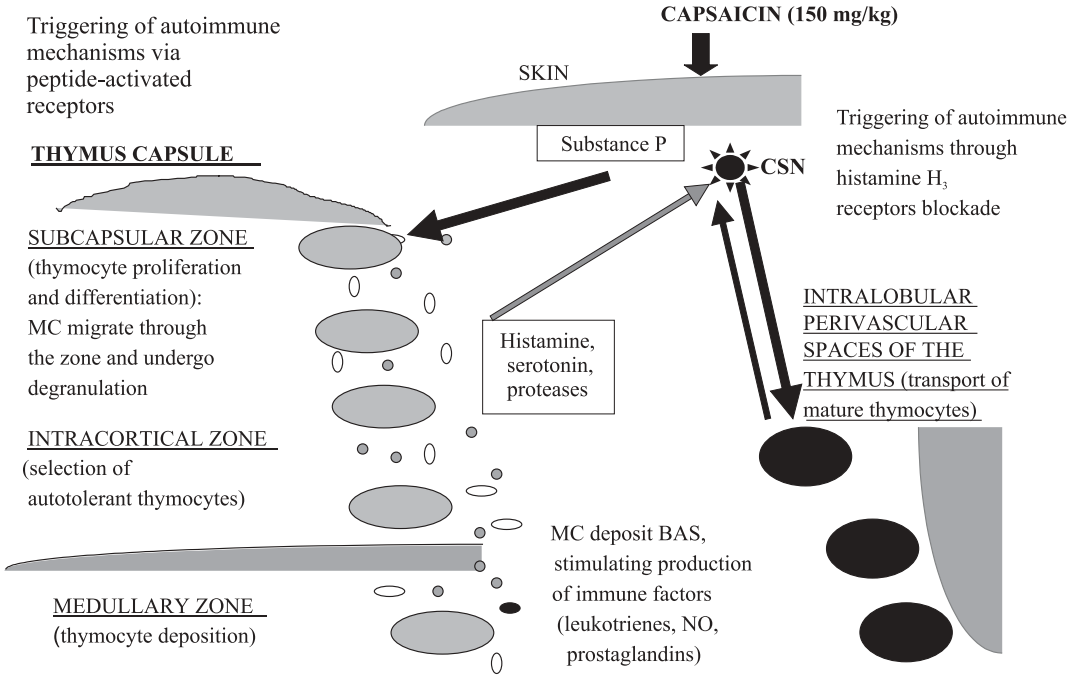

Fig. 3. Effect of capsaicin deafferentation on the reaction of mast cells (MC) in Wistar rats. CSN: capsaicin-sensitive neurons; BAS: bioactive substance.

phenomenon is primary release of neuropeptides. It is known that synthetic analog of substance P activates (through membrane G proteins) the synthesis of leukotrienes and TNF-α [11].

Hence, the imbalance of sensory neuropeptides after injection of capsaicin in a neurotoxic dose led to a significant decrease in the lymphoid parenchyma of the thymus and reduced its structural reserve. The morphological picture in general indicated dysfunction of the T-immunity system. Our analysis indicates that nerve terminals are an important factor of morphogenesis for the thymus. We mean here certain similarity of disorders observed in pharmacological deafferentation, chronic stress exposure, with age, and under the effect of autoantigens. These data suggest that capsaicin-sensitive neuron neuropeptides are involved in the regulation of autoimmune processes in the thymus.

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