Effect of Capsaicin on Dynamics of Neutrophil Functional Activity in Wistar Rat Venous Blood

E. M. Zhukova and O. P. Makarova*

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Capsaicin in neurotoxic dose produced phasic changes in functional activity of sensory neuropeptides and venous blood neutrophils of Wistar rats. At the early terms after capsaicin injections, the sensitivity of neutrophils to bacterial stimuli decreased against the background of neuropeptide release from the sensory terminals. After 2 weeks, activity of neutrophils assessed by NBT-test increased against the background of depletion of neuropeptide stores, while total antioxidant activity of venous plasma decreased. On day 21 of capsaicin treatment, when the content of neuropeptides returned to the baseline level, functional activity of neutrophils decreased and plasma antioxidant activity returned to the control level.

Key Words: *sensory neuropeptides; capsaicin, neutrophils; NBT-test; blood antioxidant activity*

Apart from transmission of sensory information from chemo-, thermo-, and nocireceptors capsaicin-sensitive neurons (CSN), their mediators (substance P and calcitonin-gene-related peptide) are involved in activation of specific defense reaction — a neurodegenerative process associated with enhanced vascular permeability, exudation of plasma proteins, and intensive leukocytic infiltration [6,10]. The selective antagonists of NK-1 tachykinin receptors spantid, CP-96.345, and dipeptide S18523 drastically suppress inflammatory reaction in visceral organs triggered by injection of substance P [15]. It was hypothesized that the phlogogenic effect of substance P is mediated by activation of monocytes and granulocytes playing the key effector role in inflammation [3]. There are data on interaction of neuropeptides with mononuclear phagocytes. Substance P up-regulates production of superoxide radicals, thromboxanes, lysosomal enzymes, prostaglandins, leukotrienes, interleukin-1, and tumor necrosis factor by cultured macrophages [11]. The activating effect of neuropeptides can be explained by the presence of NK-1 and NK-2 tachykinin receptors on macrophage membrane [7].

The role of polymorphonuclear phagocytes in the realization of the effects of neuropeptides was not studied. Neutrophils also possess receptors to neuropeptides, injection of 1% capsaicin into the knee-joint induces intensive neutrophilia of the synovial fluid [13]. It was also shown that neuropeptides potentiate neutrophil adhesion to venous endotheliocytes [7]. Capsaicin is an important tool in the studies of the physiological role of sensory neuropeptides. Capsaicin in neurotoxic doses disturbs the balance between sensory neuropeptides: the initial intensive release is followed by depletion of their depots in sensory terminals [10]. The release of neuropeptides induced by a toxic dose of capsaicin can provoke essential changes in the blood. In light of this we studied how the functional state of CSN affects biocidal activity of venous neutrophils and the total antioxidant potential of the plasma.

MATERIALS AND METHODS

Pharmacological deafferentation was performed as follows: 1% capsaicin (Serva) dissolved in a mixture

Laboratory of Functional Neuromorphology, Institute of Physiology; *Laboratory of Pathophysiology, Institute of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** E.V.Zhukova@iph.ma.nsc.ru. Zhukova T. M.

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10% ethyl alcohol, 10% twin-80, and 80% physiologic saline was injected subcutaneously to mature Wistar rats under light ether narcosis in the doses of 25, 25, 50, 50 mg/kg for 2 days with 12-h intervals. The total neurotoxic dose was 150 mg/kg. Blood samples were taken on days 7, 14, and 21 after capsaicin treatment. Production of biooxidizers by neutrophils was assessed by spontaneous and induced [4] NBT reduction. For evaluation of spontaneous production 25 μl Hank's solution and 25 μl 0.2% NBT solution were added to 50 μl heparinized (10 U/ml) blood. For evaluation of induced production the blood was incubated with 25 μl inductor solution (instead of Hank's solution) for 30 min at 37°C. The inductors were prodigiosan (LPS from *Serratia marcescens*) and killed *St. aureus* vaccine. The total and differential leukocyte counts were determined routinely. Neutrophils reducing NBT into dark-blue NBD (Nitro Blue Diformazan) were counted under a light microscope at ×1000.

Antioxidant activity (AOA) of blood plasma induced by H_2O_2 was recorded as follows. Freshly isolated plasma samples (0.1 ml) and 0.8 ml dye-free Hank's solution were placed into a measuring cell and thermostated at 37°C for 5 min. H_2O_2 (0.1 ml, final concentration 3%) was added to the sample and after 20 sec chemiluminescence was recorded. Plasma AOA was determined as a reciprocal value to chemiluminescence intensity and expressed in relative units. This method and its modifications were tested in clinical and experimental studies [1,2]. Chemiluminescence was recorded on an SKIF-0306 chemiluminometer (Nauka Co.) [5].

RESULTS

During the first week of systemic neurodegenerative process, activity of neutrophil population remained virtually unchanged, but the sensitivity of these cells to microbial stimuli markedly decreased. Parameters of NBT-test with prodigiosan or killed staphylococcus vaccine decreased 1.6- and 1.4-fold, respectively, compared to the control (Fig. 1). The decrease in neutrophil reactivity can be caused by neuropeptides released from the peripheral neural stores in primary sensory terminals.

On day 14 after capsaicin injections, when neuropeptide stores in sensory terminals were depleted, activity of peripheral blood neutrophils assessed by spontaneous NBT-test increased 2-fold compared to the control ($p<0.01$). In tests with stimulation with prodigiosan or killed staphylococcus vaccine their activity increased 1.4- and 2-fold, respectively (Fig. 1).

The increased leukocyte count in venous blood observed 2 weeks after injection of neurotoxic dose of capsaicin was determined by lymphocytes, because the

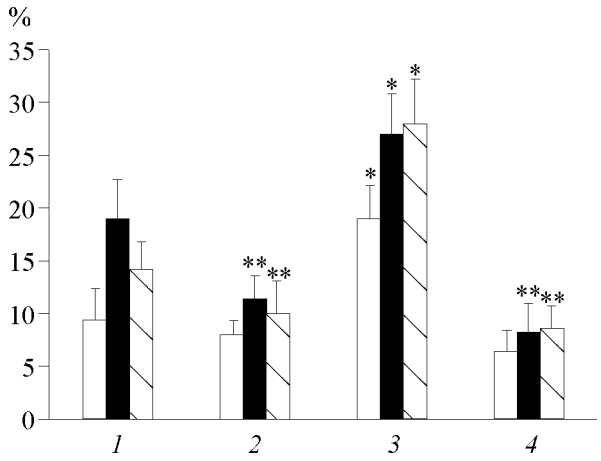

Fig. 1. Percentage of NBT-positive neutrophils in rat blood in control (*1*) and on days 7 (*2*), 14 (*3*), and 21 (*4*) after capsaicin treatment. Spontaneous, prodigiosan-, and staphylococcus vaccine-induced NBT-tests are shown by open, solid, and dashed bars, respectively. **p*<0.01, ***p*<0.05 compared to the control.

count of neutrophils significantly decreased (2.70±0.47×10^9 *vs.* 5.20±0.60×10^9/liter in the control; $p<0.01$). The percentage of lymphocytes in experimental rats surpassed the corresponding parameter in controls (72.00±6.27 and 59.00±3.26%, respectively, $p<0.05$); the percentage of neutrophils was lower than in controls (28.0±5.9% and 39.00±3.42%, respectively, $p<0.05$).

Total AOA of venous plasma on day 14 after capsaicin treatment decreased 1.6-fold in comparison with the control ($p<0.05$, Fig. 2). At this term, the decrease in total plasma AOA was accompanied by enhanced production of biooxidizers by polymorphonuclear leukocytes and probably resulted from inactivation of sensory neuropeptides after injection of capsaicin in neurotoxic dose. It is known that neuropeptides in nanomolar concentrations produce a priming effect on the neutrophils, stimulate chemotaxis and cytotoxicity, and modulate the inflammatory response.

On day 21 after capsaicin treatment, the percentage of NBT-positive neutrophils in spontaneous test returned to the control level, while in the test with inductors this parameter decreased below the control: by 2.3 and 1.6 times in tests with prodigiosan and killed staphylococcus vaccine, respectively ($p<0.05$, Fig. 1). No significant changes in plasma AOA were observed under these conditions.

Stimulation of neural sensory terminals with capsaicin changes the content of substance P in these terminals and in the blood and increases venous and capillary permeability [10,12]. Damage to the microcirculatory bed produced by substance P-activated neutrophils is inhibited by the blood antioxidant system. This process depletes the pool of active antioxidants. Therefore, pronounced activation of neutrophilic leukocytes

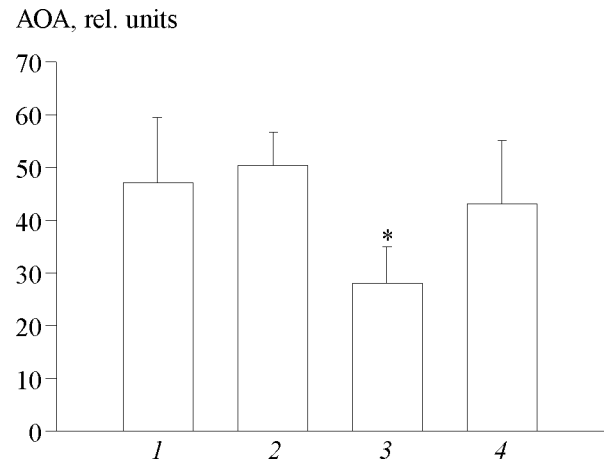

Fig. 2. Antioxidant activity (AOA) of Wistar rat venous plasma in control (*1*) and on days 7 (*2*), 14 (*3*), and 21 (*4*) after capsaicin treatment. *$p<0.05$ compared to the control.

in peripheral blood (revealed by spontaneous NBT-test) related to hyperproduction of reactive oxygen metabolites can moderate plasma AOA.

Phasic changes in functional activity of neutrophils and antioxidant potency of the plasma triggered by capsaicin correspond to changes in neuropeptide content in CSN terminals. Phagocytizing cells, the effectors and modulators of the inflammatory process, probably participate in the realization of physiological function of neuropeptides of CSN as effectors of spinal centers vascular regulatory. It can be assumed that massive release of neurogenic factors (neuropeptides) activates tachykinin receptors in neutrophils and induces local defense circulatory and tissue reaction directed at restoration of homeostasis. The effect of manual therapy is explained by "respiration burst" of neutrophils caused by the release of substance P [12]. Substance P stimulates production of superoxide anion by neutrophils [7]. Depletion of neuropeptides in peripheral terminals of CSN after 2-week capsaicin treatment leads to a decrease in plasma AOA.

Degeneration of primary sensory neurons can be an important component of inflammatory pathologies. This process accompanies initial stages of various diseases. CSN transmit the signals of noxious and heat stimuli to CNS. Functional activity of CSN can be assessed by the efficiency of afferent traffic, while their local efferent function can be evaluated by the reaction of tissue acceptors of their transmitters (neutrophils). These observations should be taken into consideration in medical practice during the development of complex methods of clinical diagnostics.

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