Stress-Induced Changes in Cellular Responses in Hypothalamic Structures to Administration of an Antigen (Lipopolysaccharide) (in Terms of C-Fos Protein Expression)

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Stress is known to affect the intensity of the immune response. The involvement of central regulatory structures in mediating these changes was addressed by analyzing the extent of activation of neurons in the hypothalamus (in terms of the number of c-Fos-positive cells) in rats 2 h after i.v. administration of lipopolysaccharide alone and on the background of electrical pain stimulation. Studies were performed using 52 male Wistar rats weighing 200–250 g. c-Fos protein expression was studied by immunohistochemical analysis. Increases in the quantity of c-Fos-positive cells 2 h after administration of lipopolysaccharide were seen in the following hypothalamic structures: AHN, PVH, LHA, VMH, DMH, and PH. After electrical pain stimulation, the number of c-Fos-positive cells increased in these same hypothalamic structures (AHN, PVH, LHA, VMH, DMH, and PH). The combination of electrical pain stimulation and lipopolysaccharide administration led to a decrease in the extent of activation in hypothalamic structures AHN, PVH, LHA, and VMH as compared with the characteristic reaction to lipopolysaccharide without electrical pain stimulation. Electrical pain stimulation suppressed the intensity of the immune response induced by lipopolysaccharide (as assessed by local hemolysis and counts of the numbers of spleen antibody-forming cells). Thus, changes in the extent of activation of hypothalamic structures (AHN, PVH, LHA, VMH) correlated with the development of stress-induced immunosuppression, i.e., morphofunctional mapping of the extent of activation of hypothalamic structures allowed identification of which changes in hypothalamic cell activity occurred with stress-induced changes in immune system responses to antigen administration.

KEY WORDS: hypothalamus, c-Fos gene expression, electrical pain stimulation, lipopolysaccharide.

Various stressful treatments induce a wide complex of functional changes, including rearrangements of the pattern of neuron activation in the hypothalamus. Electrical pain stimulation in rat is followed by decreases in the cytotoxic activity of spleen natural killer cells [23], while administration of lipopolysaccharide (LPS) on the background of electrical pain stimulation is followed by decreases in lymphocyte counts, interleukin-1, and tumor necrosis factor in the spleen [19, 21], i.e., there is an immunosuppressive effect. On the other hand, administration of antigens induces neuron activation in various brain structures, particularly the hypothalamus, which is involved in regulating immune system functions. A large volume of data has now been accumulated to provide evidence of increases in c-Fos protein synthesis (a marker of cell activation) in brain structures, including the hypothalamus, in response to a variety of stimuli [1, 6, 8, 13]. I.v. administration of lipopolysaccharide increases the number of c-Fos-positive cells in a number of hypothalamic structures in rats [11, 15, 25].

Clinical observations provide evidence of an increased incidence of more or less severe courses of infectious pro-
cess in patients on the background of stress. Increases in the level of infection of mice with herpes simplex have been observed after electrical pain stimulation [12]. However, many of the mechanisms of the influences of stress on immune system function are unclear.

The aim of the present work was to study the central effects of electrical pain stimulation on lipopolysaccharide-induced activation of neurons in hypothalamic structures by analyzing changes in the numbers of c-Fos-positive cells in rat hypothalamic structures after combined use of electrical pain stimulation and lipopolysaccharide administration.

METHODS

Experiments were performed on 52 male Wistar rats weighing 180–220 g. Animals were kept in animal-house conditions at room temperature with a 12-h light:dark cycle, free access to water and food, and a standard diet, in accord with the norms for keeping laboratory animals. Experimental animals were acclimated to the experimental apparatus for five days: rats were placed in plastic cylindrical containers corresponding to the size of the animal at 10:00 each day for 1 h.

Experimental animals. Seven animals were used in each experiment.

1. An intact animal in standard animal-house cage conditions – control 1.
2. An animal adapted to the experimental conditions, placed for 60 min in the plastic cylinder with attachment of electrodes to the knee joints – control 2 (sham electrical pain stimulation).
3. An animal adapted to the experimental conditions and, 60 min after, being placed in the container, given i.v. physiological saline – control 3.
4. An animal adapted to the experimental conditions and subjected to electrical pain stimulation for 60 min and, 1 h after the end of electrical pain stimulation, given i.v. physiological saline – control 4.
5. An animal adapted to the experimental conditions, subjected to electrical pain stimulation for 60 min.
6. An animal adapted to the experimental conditions and, 60 min after being placed in the container, given i.v. lipopolysaccharide.
7. An animal adapted to the experimental conditions and subjected to electrical pain stimulation for 60 min and, 1 h after the end of electrical pain stimulation, given i.v. lipopolysaccharide.

Electrical pain stimulation was performed via electrodes consisting of steel helices (diameter of intravenous helical loops, \(d = 2.0 \text{ mm} \), total length of helix, \(l = 150 \text{ mm} \), diameter of steel wire from which helices were made, \(d' = 0.1 \text{ mm} \)), which were placed on the skin at the knee joint. The magnitude of the direct current electrical impulse was 2.5 mA, its duration was 1 sec, and shocks were delivered at a rate of 10 per min in random order for 60 min.

Antigen consisted bacterial lipopolysaccharide (Sigma, L-2880) at a dose of 25 \(\mu g/kg \) in 200 \(\mu l \) of physiological saline (0.9% NaCl). Controls were intact, adapted animals, as well as animals given injections of apyrogenic physiological saline (200 \(\mu l \)).

Animals were anesthetized with Nembutal (60 mg/kg) 60 min after i.v. administration of lipopolysaccharide or apyrogenic physiological saline (into the tail vein) or 180 min after electrical pain stimulation; chests were opened and animals were subjected to intracardiac perfusion with 50 ml of warm physiological saline containing heparin (10 U/ml) at a flow rate of 10–15 ml/min. This was followed by perfusion with 250 ml of fixative solution: 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer pH 7.4.

Extracted brains were post-fixed with a new portion of fixative mixture at +4°C for 12 h.

Sections of thickness 20 \(\mu m \) were prepared using a microtome (Reichert, Austria) and a freezing platform (Mikonta 02, Russia). Sections were washed three times (5 min each) with 0.01 M phosphate-buffered saline pH 7.4 at room temperature to remove excess fixative and were placed in 3% \(\text{H}_2\text{O}_2 \) solution; sections were again washed three times (5 min each) in phosphate-buffered saline. Sections were then incubated in solution containing 1% bovine serum albumin in phosphate-buffered saline and 0.4% Triton X-100 for about 1 h to prevent nonspecific binding of antibodies, washed in phosphate-buffered saline three times (5 min each), and incubated with rabbit antibody to anti-c-Fos protein (Sigma, 1:5000, Lot 079H 4859) (0.1% BSA, 0.01 M PBS, 0.4% Triton) for 12 h at room temperature. The fact that antibody to human c-Fos protein can be used to detect rat brain c-Fos protein results from the high degree of homology of the rat and human c-Fos proteins. After washing (three times, 5 min each) in 0.01 M phosphate-buffered saline, sections were incubated with anti-rabbit antibody (Sigma, 1:300) (0.01 M h-buffered saline, 0.4% Triton) for 1–2 h, washed with 0.01 M phosphate-buffered saline (two times, 5 min each) and 0.01 M Tris-HCl pH 7.4 (once), and stained for 5–15 min in 0.02% diaminobenzidine solution in 10 mM Tris-HCl buffer pH 7.4 containing 0.001% \(\text{H}_2\text{O}_2 \).

Stained sections were mounted in slides and permanent preparations were made by coating slides with Canada balsam.

Controls consisted of sections prepared for: 1) histological staining (to characterize brain topography and identify the total number of cells per unit area of sections); 2) immunohistochemical staining a) to control for endogenous peroxidase, and b) to control for the specificity of binding of secondary antibody (primary antibodies were omitted).

Cells were counted using the Ista-Vidio-Test system (St. Petersburg). Frontal brain sections were examined