EXPERIMENTAL BIOLOGY

Effect of Transection of Various Branches of the Vagus Nerve on Lipopolysaccharide-Induced Synthesis of Corticotropin-Releasing Hormone mRNA in the Paraventricular Nuclei of Rat Hypothalamus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 7, pp. 106-109, July, 2000
Original article submitted January 5, 2000

Effects of selective transection of the gastric, celiac, and hepatic branches of the vagus nerve on expression of corticotropin-releasing hormone mRNA in small cell neurons of the hypothalamic paraventricular nuclei in rats administered with bacterial lipopolysaccharide were studied using *in situ* hybridization technique. Low doses of lipopolysaccharide stimulated expression of corticotropin-releasing hormone mRNA in rats subjected to axotomy of the gastric or celiac branches of the vagus nerve, but did not change the intensity of autoradiographic labeling in animals with transected hepatic branches. High doses of lipopolysaccharide enhanced expression of corticotropin-releasing hormone mRNA in vagotomized rats of all groups, which indicated the existence of a vagus-independent mechanism responsible for activation of paraventricular neurons mediating the effect of this hormone. The data suggest that the inflammation-dependent activation of stress-regulating neurons in the hypothalamus is controlled by several mechanisms, whose activation depends on the severity of inflammatory processes.

Key Words: *corticotropin-releasing hormone; vagus nerve; inflammation; hypothalamic paraventricular nuclei*

Invasion of pathogens induces a variety of adaptive reactions in the body, including fever, vigilance, and activation of the hypothalamic-pituitary-adrenal axis [12], which are controlled by the central nervous systems. At the same time, it is unknown how peripheral signals characterizing impaired immune functions are transmitted to the brain. It was hypothesized [7] that inflammatory signals are transmitted into the brain via neuroconductive pathways.

This theory postulates that the vagus nerve conveys inflammatory impulses from the abdomen. Previous experiments showed that bilateral subdiaphragmatic vagotomy attenuates depression of social activity [3] and fever [11] produced by peripheral injection of bacterial lipopolysaccharide (LPS). Desensitization of chemosensory fibers of the vagus nerve by intraperitoneal administration of capsaicin suppresses LPS-induced fever [16], which indicates that pyrogenic signals are transmitted into the brain through vagal afferent fibers. Since afferent fibers of the abdominal vagus nerve are present in its celiac, gastric, and hepatic branches, the question arises on the contribution of each branch into transduction of the inflammatory signals.

Previous studies of the role of various vagus nerve branches in mediation of LPS-induced fever showed

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0007-4888/00/0007-705$25.00

that pyrogenic signals are transmitted to the brain primarily through afferent fibers of the hepatic branch [14]. However, the contribution of afferent innervation of the vagal hepatic branch in other central components of reactive inflammation (e.g., activation of stress-regulating nerve structures) remains unclear. The synthesis of corticotropin-releasing hormone (CRH), a key mediator of the stress response, by the hypothalamic paraventricular nuclei (PVN) reflects activation of the nervous system [8].

The data on the role of visceral afferents in mediation of inflammation-induced central reactions are ambiguous. Some authors believe that the vagus nerve is involved in transduction of inflammatory signals to the brain, while others reported that vagotomy has no effect on LPS-induced reactions [9,10]. These contradictory results are probably due to the use of various doses of LPS. This problem requires further comparative studies.

To evaluate the role of the vagus nerve in transduction of LPS-induced inflammatory signals and to analyze the contribution of various vagal branches in the development of stress reactions to infection, we studied the effects of various doses of LPS on the intensity of CRH synthesis in hypothalamic PVN neurons of rats subjected to selective transection of the gastric, celiac, and hepatic branches of the vagus nerve.

MATERIALS AND METHODS

Experiments were performed on 48 male Sprague-Dawley rats weighing 300-350 g. The rats were kept under standard light-dark cycle (day-time 6.00-18.00) at 22°C and *ad libitum* food and water supply. Four weeks before experiment, the celiac, gastric, or hepatic branches of the vagus nerve were transected. Each experimental group included 12 rats. The animals were anesthetized with Nembutal (60 mg/kg intraperitoneally), and longitudinal laparotomy was performed. The liver, stomach, and the lower portion of the esophagus were removed from the abdomen and covered with physiological saline-impregnated sterile cloth. Under microscope, the nerve trunks were separated from the esophageal serous membrane, and the anterior and posterior gastric or celiac branches of the vagus nerve were transected. The unpaired hepatic branch was transected 5 mm distally to the site of its branching from the esophagus. After surgery the organs were placed into the abdomen, and cuts were sutured. Twelve sham-operated rats served as the control.

Twenty-eight days after surgery, vagotomized and sham-operated animals (4 rats per group) were intraperitoneally injected with low (5 μg/kg) or high (125 μg/g) dose of LPS (Sigma) in 1 ml 0.9% NaCl, or with sterile physiological saline. Four hours postinjection the rats were decapitated, and the brain was rapidly removed and frozen for *in situ* hybridization. Hypothalamic slices (14 μ) were mounted on slides (Fisher Sci), dried, and incubated with a radiolabeled probe complementary to 1-34 nucleotide sequences in rat CRH mRNA (Scandinavian Gene Synthesis) at 42°C for 16 h. The probe was diluted in a solution containing 50% formamide, 0.015 M citrate buffer, 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 1% N-lauroylsarcosine, 10% dextran, 500 mg/liter denatured DNA from salmon testes, and 200 mM dithiothreitol. After hybridization, the slices were washed with citrate buffer at 55°C, dried on air, and covered with an Ulford emulsion. After 3-week exposure, the slices were developed and fixed in Kodak reagents and then stained with toluidine blue. The number of silver grains above labeled neurons was

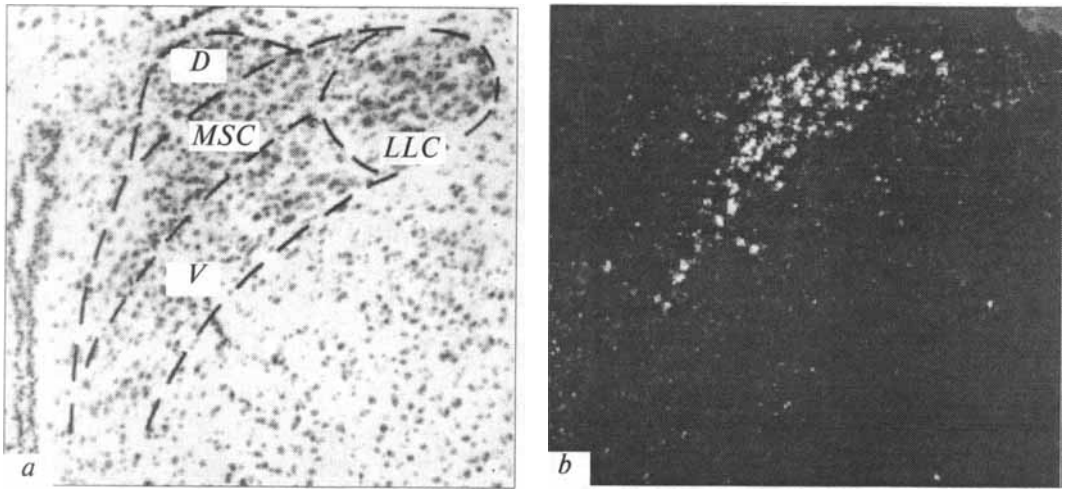

Fig. 1. Localization of neurons expressing corticotropin-releasing hormone mRNA in rat hypothalamic paraventricular nucleus. The same nuclei in light (*a*) and dark (*b*) fields. Dorsal (*D*), medial small cell (*MSC*), ventral (*V*), and lateral large cell (*LLC*) subnuclei.

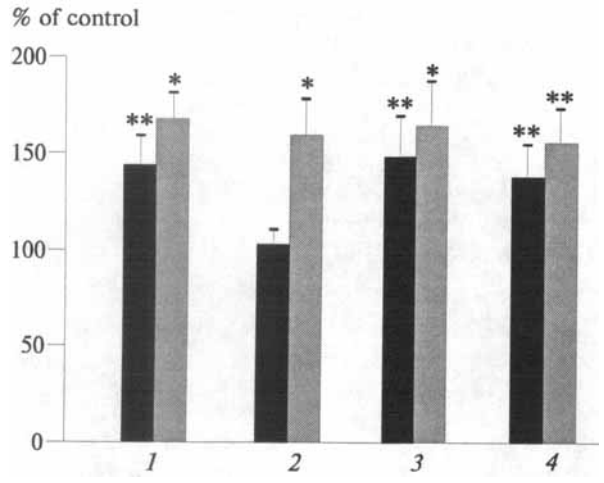

Fig. 2. Effects of low (5 μg/kg, dark bars) and high (125 μg/kg, shaded bars) doses of LPS on expression of corticotropin-releasing hormone mRNA in neurons of the paraventricular nucleus in sham-operated rats (*1*) and after transection of the hepatic (*2*), gastric (*3*), and celiac branches (*4*) of the vagus nerve. Ordinate: the mean number of silver grains above cells. *$p<0.01$ and **$p<0.05$ compared to the control (100%).

calculated using a Nikon Microphot-FX microscope equipped with a dark-field condenser. The neuron was considered to be labeled, if the number of silver grains 5-fold surpassed that in the control. We calculated no less than 200 cells in hypothalamic slices from each rat. The results were analyzed by Student's *t* test.

RESULTS

The autoradiographic label corresponding to binding sites of the probe complementary to CRH mRNA was found above neurons of the medial small cell subnucleus of PVN (Fig. 1). Administration of LPS dose-dependently increased synthetic activity of CRH-ergic neurons in sham-operated rats compared to that in animals injected with physiological saline (by 44, $p<0.05$, and 68%, $p<0.01$), respectively (Fig. 2).

Low doses of LPS had no effect on labeling intensity in PVN neurons of rats after transection of the hepatic branches, but markedly enhanced the autoradiographic signal in PVN of animals with transected gastric or celiac branches of the vagus nerve ($p<0.01$). The stimulation of CRH mRNA synthesis in rats with transected gastric or celiac branches of the vagus nerve was comparable with those in sham-operated animals injected with endotoxin. These data indicate that the hepatic branch of the vagus nerve plays the major role in activation of stress-regulating PVN neurons after intraperitoneal injection of LPS in low doses. Therefore, the initial changes in the liver probably activate afferent fibers of the vagus nerve. Kupffer cells are the most probable source of stimulatory signals, because selective chemical inactivation of these cells prevents LPS-induced fever and hyperalgesia [13]. It is known that afferent hepatic fibers of the vagus nerve originate from the periportal parenchyma [1] containing many Kupffer cells. Potentially pathogenic materials can enter the portal circulation and migrate from the gastrointestinal tract to the periportal parenchyma.

Considering central organization of the vagus nerve-dependent mechanisms responsible for activation of CRH-ergic neurons, it should be emphasized that the vagus nerve has sensory projections to solitary nucleus neurons projecting to PVN [5]. Recent studies showed that ascending catecholaminergic projections from nuclei of the solitary tract and brainstem stimulate CRH synthesis by PVN neurons after peripheral administration of LPS or cytokines [4,6].

High doses of LPS administered to rats after selective vagotomy increased the intensity of autoradiographic labeling above PVN neurons, which was similar to that in sham-operated animals ($p<0.01$). Enhanced synthesis of CRH mRNA in PVN neurons of rats with transected hepatic branches of the vagus nerve induced by high doses of LPS suggests that severe infections stimulate vagus-independent mechanisms of activation of these neurons, in particular cytokine influx across the blood-brain barrier in the region of circumventricular organs [2,15]. This region includes the *area postrema* adjacent to nuclei of the solitary tract and having projections to them. Activation of the solitary nuclei probably stimulates mRNA synthesis in vagotomized animals treated with high doses of LPS.

Our findings indicate a key role of the hepatic branch of the vagus nerve in the activation of stress-regulating hypothalamic neurons in animals receiving injections of low doses of LPS. Furthermore, severe inflammation activates vagus-independent mechanisms stimulating synthetic activity of these neurons. These data suggest that activation of stress-regulating hypothalamic neurons is controlled by several mechanisms, whose activation depends on the severity of inflammatory processes.

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