STRUCTURAL AND FUNCTIONAL CHANGES IN THE INNERVATION OF LYMPHOID ORGANS IN RESPONSE TO ELECTRICAL STIMULATION OF THE HYPOTHALAMUS

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KEY WORDS: hypothalamus; immunity; lymphoid organs.

Recent investigations have revealed various disturbances of the protective functions of the body during local procedures directed toward the hypothalamus [9, 11, 12, 14]. In particular, injury to the posterior hypothalamic area causes changes in the surface structure of macrophages, disturbances of antibody production by their lysosomal enzymes, disturbances of cooperation between macrophages and lymphocytes, delay of the plasma-cell reaction, and a decrease in the number of antibody-forming cells in the immune response to injection of various antigens [3-5, 13]. There are reports in the literature of the rich efferent innervation of lymphoid organs [2, 8, 10, 15], but the function of this innervation has not been adequately studied. An understanding of the character of neurogenic influences on immunity requires investigation of the final link in the chain of their realization: the time course of structural and functional parameters of the intramural innervation during the action of various procedures on central structures.

The aim of this investigation was to study structural and functional changes in the innervation of the lymphoid organs in response to stimulation of the posterior hypothalamic area.

EXPERIMENTAL METHOD

The intramural innervation of the mesenteric lymph nodes, spleen, and thymus of 28 rabbits was investigated after 15 and 30 dailys (1 h) sessions of electrical stimulation (0.05 nA, 1 msec, 50 Hz) of the posterior hypothalamic area through stereotaxically implanted bipolar nichrome electrodes with a cross-section 200 μ in diameter. The electrodes were inserted 7 days before stimulation began. This procedure provides an adequate model of development of reactive and, to some extent, degenerative changes in nerve cells and neurotrophic changes in tissue cells of certain internal organs [1, 6]. The experiment group comprised 14 animals (eight rabbits with 15 sessions and six rabbits with 30 sessions of stimulation). The control consisted of 14 animals: nine intact and five with implanted electrodes. The rabbits were killed by one-stage division of the common carotid arteries and jugular veins. The location of the uninsulated tips of the electrodes was verified histologically. Nerve cells were demonstrated by the histochemical methods of Sakharov (in Shustova's modification) and Karnovsky and Roots. The Bielschowsky–Gross silver impregnation method also was used. Activity of the nerve cells was assessed cytophotometrically by measuring the intensity of luminescence of adrenergic fibers and the optical density of fibers containing acetylcholinesterase (AChE). The diameter of the probe on the preparation did not exceed 15 μ. The results were subjected to statistical analysis, using 25 measurements for each experiment–control pair, and Student's paired t test. No significant differences were found between values obtained in the two groups of control animals.

EXPERIMENTAL RESULTS

Adrenergic fibers enter the lymph nodes mainly with blood vessels and form plexuses with infrequent bush-like arborizations among the reticular cells. A significant decrease in luminescence was observed after

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TABLE 1. Intensity of Luminescence of Adrenergic Nerve Fibers and Optical Density of AChE-Containing Nerve Fibers of Mesenteric Lymph Nodes after Electrical Stimulation of Posterior Hypothalamic Area (in % of control)

Structures	Control	15 sessions of stimulation	Significance of differences ($P < 0.05$)	Control	30 sessions of stimulation	Significance of differences ($P < 0.05$)
Adrenergic	100±20	78±15	+	100±9	38±10	+
	100±17	119±11	+	100±15	61±20	+
	100±4	41±7	+	100±6	76±9	+
	100±7	93±6	—	100±7	79±5	+
	100±5	67±13	+	100±18	About 0	+
				100±12	57±14	
AChE-containing	100±8	83±10	—	100±14	About 0	+
	100±6	69±5	+	100±8	About 0	+
	100±4	80±5	+	100±6	60±10	+
				100±9	91±5	—

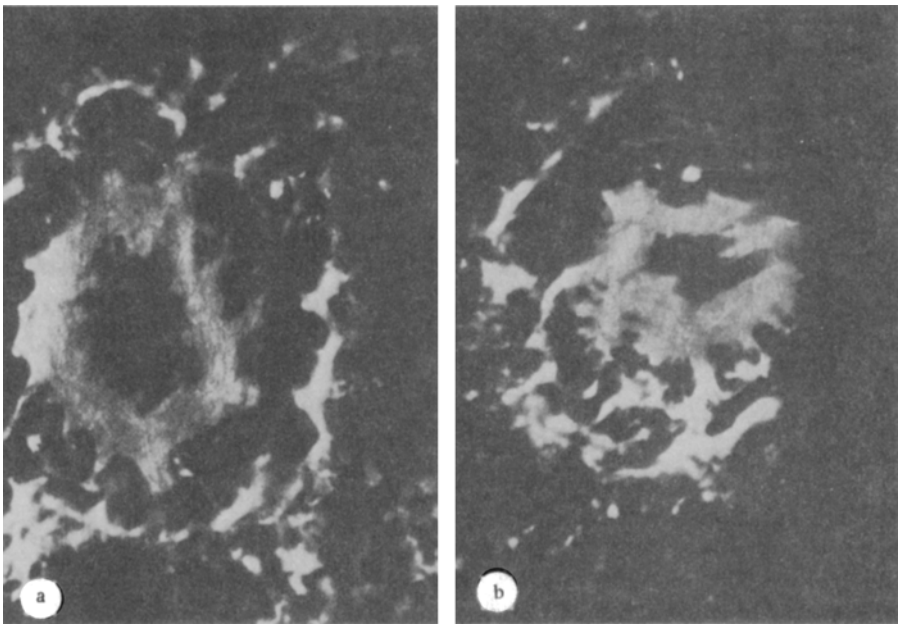

Fig. 1. Adrenergic neuronal structures of the central artery of the spleen of intact and experimental rabbits: a) control; b) experiment: 15 sessions of stimulation of posterior hypothalamic area. 60×.

15 sessions in three of the five rabbits and after 30 sessions in all six rabbits studied. AChE-containing structures in the lymph nodes formed a powerful plexus in the region of the hilus and of perivascular fibers. Activity of the latter was significantly reduced after 15 sessions in two of three rabbits, and after 30 sessions in four of five animals (Table 1).

Adrenergic fibers in the spleen form dense plexuses around blood vessels and inside their adventitia. Derivatives of these plexuses penetrate into the parenchyma, to form a sparsely looped plexus in the subcapsular zone. Changes in the intensity of luminescence during stimulation were a combination of a decrease in the perivascular plexuses, especially in the adventitia of the central arteries (Fig. 1a, b) in three of the five animals after 15 sessions and in four of the five animals after 30 sessions, of between 8 and 49%, and a simultaneous increase in activity of the fibers in the parenchyma of all the experimental animals. AChE-containing structures were connected mainly with blood vessels, but they could be found also in the parenchyma, which is particularly interesting because of disagreements in the literature [8, 10]. No unambiguous conclusion could be drawn on the state of the AChE-containing structures of the spleen during stimulation, because the changes differed in direction in different animals.

Components of the adrenergic innervation of the thymus were concentrated mainly in the perivascular plexuses, which attained a particularly high density in the subcapsular zone, and also inside the lobules of the

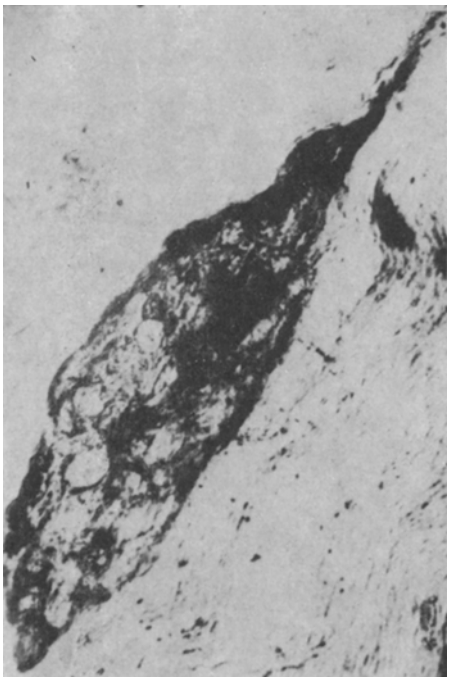

Fig. 2. Accumulation of ACh-containing neurons between capsule of thymus and large nerve trunk in a control rabbit (50×).

parenchyma, where the adrenergic fibers formed a large-looped plexus [2, 15]. No penetration of fibers was observed into the inner part of the thymic corpuscles, in our material. After 15 sessions there was a significant decrease in the intensity of luminescence by 16-36% in three animals, and a significant increase by 45% in one experimental animal, but after 30 sessions a significant decrease in the intensity of luminescence by 30% was observed in only one of five cases. Similar ill-defined changes also were observed during the investigation of AChE-containing structures, consisting of fibers and intramural neurons (Fig. 2). However, in preparations impregnated by the Bielschowsky – Gross method increased argyrophilia of the nerve cells was observed, and this is characteristic, as we know, of the reactive phase of the destructive process.

Views according to which the role of the nervous system in the regulation of immunity takes the form of a modulating action on the circulation and on specific functions and metabolism of the immunocompetent organs have recently been actively discussed in the literature [4, 9, 14, 15]. Different versions of this action may be reflex and adaptive-trophic influences. Inhibition of function of the elements of the intramural innervation of the lymph nodes and spleen and also increased argyrophilia of nerve cells in the thymus, revealed by this investigation, evidently reflect reversible changes characteristic of the early (reactive) phase of the centrogenic degenerative process [7]. Meanwhile the increase in activity of fibers responsible for the adrenergic innervation of the splenic parenchyma can be interpreted as the result of selective activation of sympathetic mechanisms of regulation of the organs of the immune system.

The reactive changes discovered are evidence of participation of the innervation of the lymphoid organs in the realization of the effects of a long-term course of hypothalamic stimulation on their function. It can be tentatively suggested that the changes observed are effected through lymphocyte membrane receptors and the cyclic nucleotide system.

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AUTORADIOGRAPHIC STUDY OF ONTOGENY OF CEREBRAL CORTICAL IMIPRAMINE RECEPTORS IN RATS

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Imipramine receptors, found initially in the rat brain, have subsequently been discovered in human brain tissue and classed with the "drug receptors" [2]. Their number has been determined in different parts of the brain and the highest density of imipramine receptors has been shown to be in the hypothalamic zone and cerebral cortex [3]. The need for an ontogenetic approach to the study of drug receptors of this type is determined by the increasingly wide use of imipramine in the clinical treatment of depressive states.

This paper describes an analysis of the number and distribution of imipramine receptors in the rat cerebral cortex during normal ontogeny and antenatal exposure to imipramine.

EXPERIMENTAL METHOD

Wistar rats were used. Imipramine was injected subcutaneously in a dose of 5 mg/kg into pregnant rats on the 17th, 18th, and 19th days of embryonic development. Three age groups were studied: 19th day of embryonic development and 3rd and 14th days of postnatal development (three animals in each group). Under pentobarbital anesthesia (60 mg/kg) the rats were perfused through the left ventricle with 0.1% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4. This procedure does not affect the binding level, while improving the histologic integrity of the preparation a little [5]. After removal of the brain, frontal slices were cut to a thickness of 2-3 mm, frozen in liquid nitrogen, and placed on the stage of a freezing microscope. Frozen sections about 25 μ thick were mounted on gelatin-coated slides and dried at 4°C. The finished preparations were kept at −20°C. A whole series of preparations was incubated simultaneously in medium containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM KCl, and 10 mM [^{3}H]imipramine (814 Tbq/millimole). The sections were incubated for 2 h at room temperature, washed with cold (4°C) buffer, and dried at 4°C. Strips of lavsan film, coated with emulsion, were glued to the slides with the sections, covered with teflon film

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