Effect of Toluene on Bioamine-Containing Structures in the Spleen

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The effect of toluene administered into the stomach on amino-containing structures in the spleen of random-bred albino mice was studied. It was shown by Falck—Hillarp method that 6 h after treatment the toxicant stimulated splenic mast cell population and inhibited other amino-containing structures. It is therefore suggested that in control mice the major role in bioamine metabolism in the spleen is played by granular fluorescent cells, while after poisoning, mast cells functioning as adapters acquire the primary role. The levels of catecholamines and serotonin in nervous and nonnervous structures peaked 1 weekafter poisoning and returned to normal after 4 weeks. Presumably, toluene suppresses the immunity starting from the second week after treatment.

Key Words: *catecholamines; serotonin; spleen; toluene*

The functional role of biogenic amines catecholamine (CA) and serotonin (ST) in animals in health and after various experimental exposures is an important problem in biology and medicine [9]. Bioamines attract great interest because of their important and unique functions in the organism. CA and ST released into the blood by neuroendocrine cells act as neurohormones, while those released from neuronal terminals in synapses act as neurotransmitters. Biogenic amines mediate the nervous regulation of various functions in the organism [6]. Bioamines are contained mainly in granular fluorescent (GFC), mast cells, and nerve fibers [4,10] and regulate the immune function [5].

Pathomorphology of the spleen was extensively studied. However, many problems, *e. g.*, the effect of toluene on the distribution of CA and ST in splenic structures and other immune and hemopoietic organs, are still unsolved and became the object of this study.

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MATERIALS AND METHODS

Experiments were performed on random-bred albino mice (25.3±1.1 g), 10 animals per control and experimental group. Toluene solution in vegetable oil was administered intragastrically in a dose of 600 mg/kg. Controls were given 0.2 ml vegetable oil. The spleens were collected 6 h, 1 day, 1 and 4 weeks after administration of the toxicant under deep ether narcosis. The studies were carried out in October—November. Cryostat sections were treated by the Falck—Hillarp method modified by E. M. Krokhina [2].

The content of CA and ST was evaluated under a LYUMAM-4 fluorescent microscope with an FMEL-1A microfluorimetric headpiece using voltmeter (600 V) with a 0.5 probe, the parameters were recorded from the voltmeter amplifier gaging part in arbitrary units. Filter 6 (480 nm) and 8 (525 nm) were used for CA and ST, respectively.

The data were processed using Statistica software [1].

RESULTS

Six hours after administration of toluene, red and white pulp was clearly seen in the spleen treated by the Falck

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method. The fluorescence spectrum of the central artery was diffuse greenish. The concentrations of CA and ST in this structure was significantly (more than 4-fold) decreased in comparison with the control (Table 1, Fig. 1, *a*).

The periarterial zone contained no GFC and mast cells and sometimes had only 1-2 fluorescent granules. The content of bioamines in periarterial T-lymphocytes was significantly decreased: 3.1 times for CA and 3.4 times for ST.

The germinal center was characterized by darker fluorescence and contained up to 6 large triangular or oval GFC with dark nuclei and yellowish granules. The content of CA and ST in these cells was more than 2-fold lower than in other zones.

In the mantle zone two types of small GFC were distinguished: with dark nuclei and fluorescent yellow granules of different size in the cytoplasm and with fluorescent granules evenly distributed in the cell (the nucleus was not seen). The content of CA and ST in GFC of both types was decreased 2.3 times in comparison with the control.

In contrast to the control, the marginal zone of lymphoid follicle contained 2 types of cells: very small round or slightly elongated GFC and mast cells. These GFC fluoresced green and the level of bioamines in them was decreased 1.8 times in comparison with the control. Mast cells were characterized by blurred green fluorescence and contained small granules of the same size.

The levels of CA and ST in the adrenergic nerves of the lymphoid follicles in vascular adventitium tended to decrease (Fig. 1, *b*).

The population of red pulp GFC was small and consisted mainly of small and medium-sized GFC with bright yellow fluorescence, sometimes with orange shade. The content of CA and ST in red pulp GFC was decreased 2.2 and 2.5 times (Table 1). GFC were distributed unevenly, with accumulations near or in contact with adrenergic nerves, which fluoresced emerald green and had decreased levels of CA (2-fold) and ST (2.5-fold) in comparison with the control.

Mast cells in the red pulp fluoresced green and had diffuse contours. The contents of CA and ST in these cells were 36.1 and 20.1 arb. units, respectively.

After 1 day the fluorescence of biogenic amines in the central artery decreased (Table 1). At this term GFC and adrenergic nerves with varicose thickenings were seen near the central artery. The content of CA and ST in the periarterial zone tended to decrease. The mean levels of CA and ST in germinal center GFC was virtually the same as in the control.

At this term the zones were not clearly discernible in the lymphoid follicles. A trend to a decrease in the content of CA and an increase in ST content were ob-

TABLE 1. CA/ST Fluorescence Intensity in Splenic Structures after Toluene Exposure (*M±m*)

Structure	Control	Term after toluene administration			
		6 h	1 day	1 week	4 weeks
Central artery	45.4±3.9/25.1±2.1	10.3±1.3*/5.8±0.6*	38.0±4.0/22.3±2.2	59.3±4.7*/31.8±2.4*	37.9±2.5/19.1±1.4*
Periarterial zone background	24.5±1.9/14.8±1.1	8.0±0.9*/4.3±0.5*	21.0±1.9/13.2±1.2	41.4±3.5*/22.4±1.4*	25.8±2.1/12.8±0.9
GFC in germinal center of lymphoid follicle	28.6±2.0/18.8±1.6	12.1±1.1*/6.3±0.5*	25.1±1.6/17.8±2.5	55.4±3.6*/30.5±1.9*	31.4±2.5/15.4±1.3
GFC in mantle zone	37.7±3.1/21.5±1.8	16.1±1.5*/9.2±1.2*	35.2±3.4/21.7±2.7	68.6±6.8*/38.9±3.6*	40.5±2.1/19.9±1.1
GFC in marginal zone	32.8±1.7/17.2±1.4	18.1±1.2*/9.2±0.6*	30.5±4.1/17.9±2.3	60.9±6.0*/32.2±1.9*	36.8±1.7/15.9±0.8
Mast cells in lymphoid follicle	21.2±4.7/12.2±3.1	19.9±0.6/10.3±0.4	28.7±3.6/16.6±2.4	—	45.5±6.9*/27.8±3.5*
	—	22.1±1.4/11.3±0.7	58.3±7.8/40.4±2.9	—	31.5±2.9/14.8±1.4
Red pulp background	29.6±4.4/16.6±2.1	17.4±2.4*/10.8±1.3*	38.7±4.2/24.6±3.1*	68.4±10.5*/37.2±4.8*	35.7±3.2/17.6±1.3
Red pulp GFC	66.5±5.7/37.6±3.0	30.5±3.0*/14.9±1.5*	73.1±10.6/43.4±5.3	97.6±5.8*/50.9±2.3*	69.8±4.7/29.8±2.4
Red pulp adrenergic nerves	81.7±7.9/45.5±4.6	41.1±3.5*/18.1±1.7*	52.9±9.4*/53.3±8.1	152.7±19.4*/81.5±8.1*	91.9±7.1/45.5±4.2
Red pulp mast cells	—	36.1±2.6/20.1±1.3	64.6±14.9/42.5±10.0	104.7±32.5/62.4±19.9	29.7±3.7/12.5±1.1

Note. *$p<0.05$ *vs.* the control.

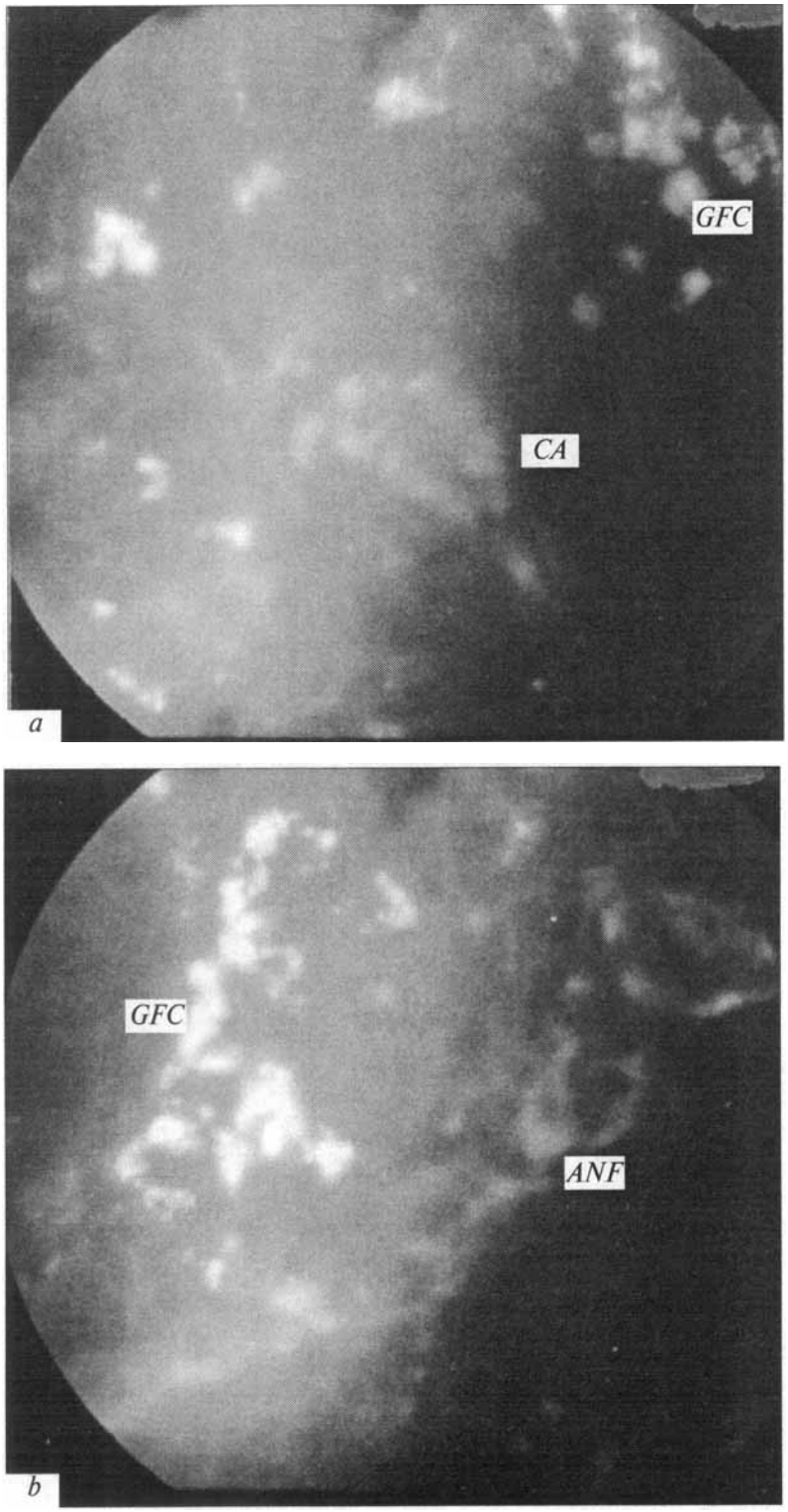

Fig. 1. Diffuse fluorescence of central artery (*a*) and bioamine-containing structures (*b*) in splenic lymphoid follicle. Falck—Hillarp staining, ×153. *CA*: central artery, *GFC*: granular fluorescent cells of the red pulp (*a*) and mantle zone (*b*), *ANF*: adrenergic nerve fiber.

served in GFC of the mantle and marginal zones. In mast cells the content of CA and ST was minimum.

The level of CA and ST in nerve fibers of the lymphoid follicle increased by 7.5 and 4.4 arb. units, respectively, in comparison with the control. The background fluorescence of the red pulp increased in comparison with the control.

In adrenergic nerve fibers the content of CA was by 28.8 arb. units lower than in the control, while the level of ST increased by 7.8 arb. units.

The increase in CA and ST levels in the red pulp GFC and mast cells was detected microfluorometrically.

After 1 week the concentrations of CA and ST in the central artery increased significantly. The periar-

terial zone contained numerous solitary granules of GFC. The background fluorescence of lymphocytes in the periarterial zone increased 1.7 times for CA and 1.5 times for ST. The germinal center GFC fluoresced only by the rim. On the other hand, the concentrations of CA and ST in them were significantly higher than in the control (1.9 and 1.6 times, respectively). In the mantle zone, GFC adhered to each other and contained significantly increased levels of bioamines. In the marginal zone the mean level of CA and ST in GFC increased 1.9 times.

Adrenergic nerves formed a dense network in the red pulp and occupied up to 2 visual fields under immersion microscope. The increase in the content of CA and ST in nerve fibers reached the peak.

In the red pulp, GFC situated mainly around nerve fibers and the levels of biogenic amines were significantly increased: 1.5 times for CA and 1.4 times for ST. Mast cells were detected only in the red pulp.

Analysis of the spleen after 4 weeks showed general diffuse fluorescence. The central artery looked like a diffuse green cloud. The concentrations of both CA and ST in this structure were decreased (Table 1).

The zones in the lymph follicle were not discernible at this last term, and therefore the cells of different zones could be recognized only structurally. The content of CA was increased and that of ST decreased in GFC of the mantle and marginal zone in comparison with the control.

Adrenergic nerves in the lymphoid follicle were seen as diffuse fine fibers in the periarterial and mantle-marginal zone. They contained 2 times more amines than in the control. Background CA fluorescence in the red pulp was increased, while ST fluorescence was similar to the control. The content of CA in GFC the red pulp tended to increase and ST level decreased *vs.* the control. The content of CA in the adrenergic nerves of the pulp increased, while ST content was the same as in the control.

Mast cells were detected only in some experimental animals, and their fluorescence was the same in the white and red pulp.

Comparison of the data of visual and fluorescent analyses demonstrated a time-effect relationship in the reaction of splenic amino-containing structures.

Toluene reduced fluorescence and decreased bioamine content in all studied splenic structures. Falck-positive fluorescent structures disappeared. Mast cells of the white and red pulp were more clearly seen in comparison with the control. Failure to detect the mast cells population in the spleen of control animals can be due to predominant bioamine metabolism through GFC, but not mast cells in mice. Toluene apparently changed bioamine metabolism in the spleen and activated mast cells. As mast cells are rapidly reacting structures, they could act as adapters [8], whose effect was directed at restoration of bioamine content in the spleen.

After 24 h the content of CA in non-nervous structures of the lymphoid follicle decreased. In GFC of the mantle and marginal zones the content of ST slightly increased; published data [4] suggest that ST inhibits blast proliferation and stimulates their differentiation in the respective zones. A similar regularity was observed in adrenergic nerves of the red pulp.

After 1 week the content of bioamines increased significantly in all splenic structures. The sympathoadrenal effects were universally directed at an increase in both CA and ST levels after toluene exposure. Visually, GFC granules appeared during this period in the periarterial zone, no clear-cut borders were seen between the mantle and marginal zones GFC. GFC completely mediated bioamine metabolism, which can explain disappearance of mast cells at this term.

After 4 weeks the homeostatic mechanisms led to a decrease in CA and ST contents in splenic amino-containing structures. The only exclusion were adrenergic nerves of the white and red pulp, where the content of amines increased *vs.* the control. Hence, exposure to toluene triggered some stress-independent mechanisms with possible predominance of the sympathoadrenal factors [7].

Hence, toluene affected mast cells and GFC and modified the immune response of the spleen to this xenobiotic.

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