Comparative Analysis of Acupuncture Effects on Amino Containing Structures of the Rat Skin in Some Acupuncture Points

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Study of the effects of 2- and 10-min acupuncture on skin structures in acupuncture points on the upper limb and trunk of outbred albino rats showed that changes in the monoamine status of the skin appeared as early as 15 min after acupuncture. However, the effects in points GV14 and LI11 were different: skin structures in acupuncture point LI11 reacted sooner and more intensively. Acupuncture caused opposite changes in the serotonin index in different structures of the skin in acupuncture points.

Key Words: *acupuncture points; mast cells; catecholamines; serotonin*

The effects of bioamines are mediated by the monoaminergic systems through mechanisms intrinsic of intracutaneous regulatory system by redistribution and activation of functionally different cell populations. Neurotransmitters and neuropeptides released from nerve endings modulate hormone secretion and cell function, including those in acupuncture points (AP). Studies by biochemical and pharmacological methods showed competitive relationships between serotonin (ST) and catecholamine (CA) in tissues [2,5]. Histochemical study of human skin revealed adrenergic nerve fibers in AP areas directly contacting with mast cells [4]. On the other hand, changes in the bioamine supply of skin structures induced by acupuncture are poorly studied.

We studied the dynamics of CA and ST levels in rat skin in various AP areas during acupuncture.

MATERIALS AND METHODS

Skin structures of 30 outbred male albino rats (180-200 g) in symmetrical AP of the large intestine and posteromedian meridians LI11 and GV14 and in zones outside these points were studied by the fluorescent histochemical method for detection of CA and ST [7]. The choice of AP was determined by the following positions. The GV14 AP is a dorsal point of general effect, LI11 AP is the distal (on the upper limb) point of general effect [1]. After location of AP, skin fragments (0.5 cm^2) were collected by an Eliteris device in AP zones; acupuncture was carried out after pre-labeling. Electrocutaneous resistance in AP varied from 58 to 65 kΩ, in control zones from 100 kΩ and higher. The intensity of pressure on the skin was the same in AP sites and adjacent skin areas. The zone located laterally from the meridian at a distance of 5 mm from each of the studied AP served as the control.

Four groups of animals were formed: group 1 comprised intact rats (n=5), in group 2 rats (n=5, controls) acupuncture was performed in zones located laterally from GV14 and LI11 AP (electrocutaneous resistance in these zones was >100 kΩ), in group 3 and 4 rats (n=20), steel needles were positioned in AP for 2 and 10 min, which corresponded to types 1 and 2 of stimulatory exposure. The material was collected, 1 h, and 24 h after the procedure under deep ether narcosis 15 min. Cryostat sections were treated by the histochemical method.

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The fluorescent histochemical method [7] modified by E. M. Krokhina [4] is based on the reaction of CA condensation with formaldehyde yielding 1,2,3,4-tetrahydroisoquinolines, which after dehydration are converted into 3,4-dihydroisoquinolines. The reaction product ketotautomers form a fluorescent complex fluorescing emerald green when exposed to visible blue-violet light. 3,4-Dihydro-p-carboline forming in these reactions from ST fluoresces yellow. The resultant preparations were examined under a LYuMAM fluorescent microscope.

Spectrofluorometry was used for quantitative expression of ST and CA levels in tissue structures of the skin. The LYuMAM-4 fluorescent microscope was fitted with a FMEL-1A attachment for this purpose. Serotonin was evaluated using a photofilter with λ=525 nm, CA with a filter with λ=480 nm. The results from the amplifier monitor were expressed in arbitrary units.

The method of serotonin index (SI) estimation was used; SI is a dimensionless value, the mean of the sum of ST and CA proportion quotients in the same cells: SI=ST/CA levels. Tissue levels for estimation of this index were measured in the same points [3]. The dynamics of the content of biogenic amines does not always adequately reflects their effects on the integral bioamine supply to the organ. Even if ST concentration increases, its effect can decrease depending on the dynamics of CA content. The ST index (reciprocity index) shows whether ST (SI>1) or CA (SI<1) predominates in the cell.

The data were statistically processed using standard software. Statistical significance of differences in the compared values was evaluated using Student's *t* test.

RESULTS

It was previously shown that the epithelium, mast cells, granular fluorescent cells, hairy follicles, adrenergic nerve fibers, elastic fibers of the reticular layer, and fibroblast nuclei served as the morpho-

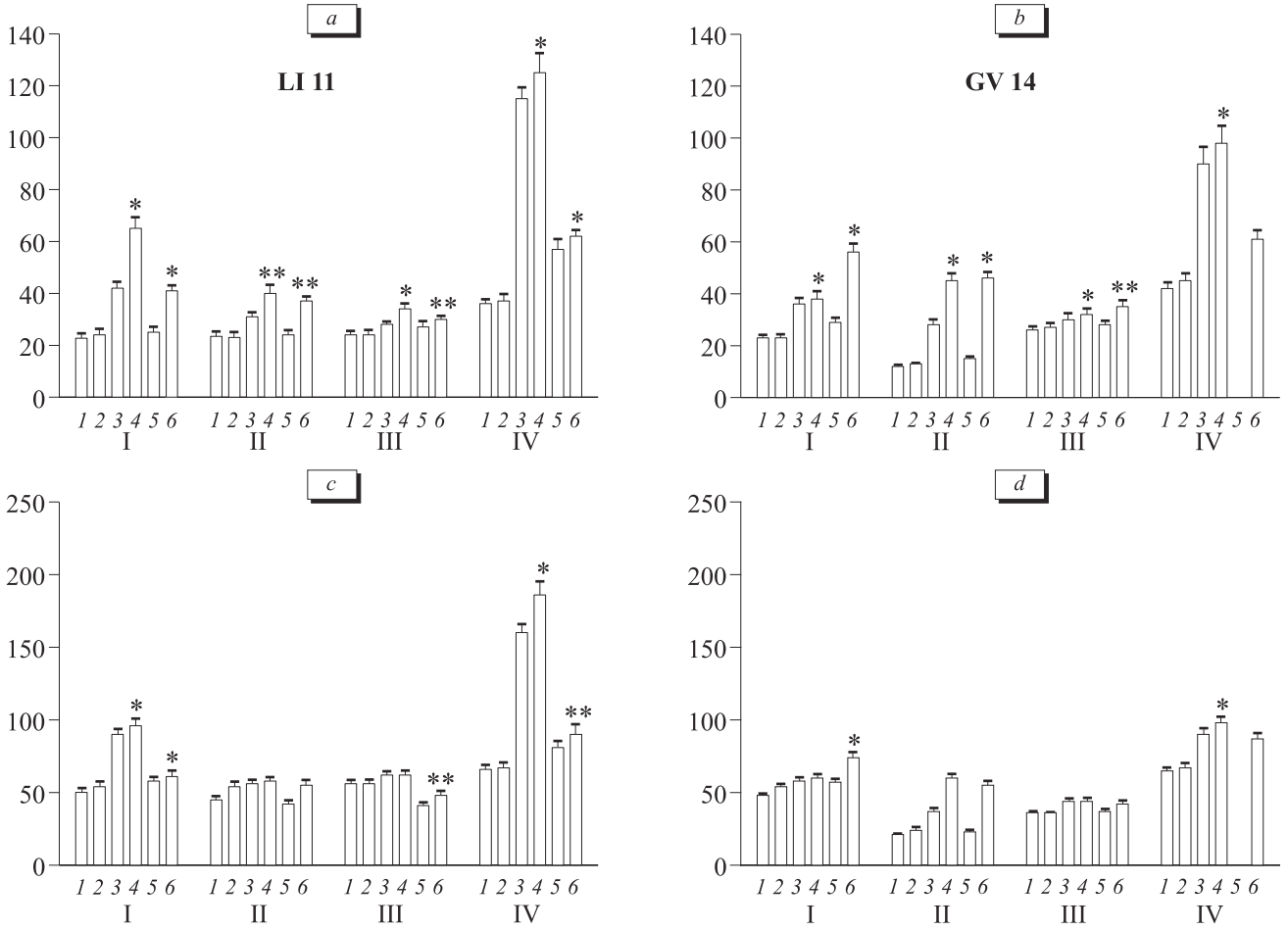

Fig. 1. Content of CA (*a*, *b*) and ST (*c*, *d*) in skin structures in AP areas after 2-min acupuncture. Here and in Fig. 2: *1*) content in control zones before acupuncture; *2*) content in AP before acupuncture; *3*) content in control zones 15 min after acupuncture; *4*) content in AP 15 min after acupuncture; *5*) content in control zones 1 h after acupuncture; *6*) content in AP 1 h after acupuncture. I) epithelium; II) hypoderma; III) subepithelial mast cells; IV) mast cells of reticular layer. **p*<0.05, ***p*<0.01 compared to the control.

logical substrate creating the bioamine provision for the rat skin in AP and adjacent areas. Fluorescent microscopy showed that by location and structure the fluorescent mast cells could be subdivided into 3 groups: 1) small oval or elongated cells under the epithelium in the dermal papillary layer characterized by diffuse fluorescence (monoamine concentration in them is low); 2) cells of different size with large yellow granuleslocated in connective tissue near hair follicles, sweat glands, and vessels; and 3) large cells with high content of bioamines with large well-discernible granules located at the interface between the dermal reticular layer and hypoderma and in the hypoderma.

Damage to the epidermis and derma after acupuncture seen as a bright yellow fluorescent strip penetrating to the hypoderma. A small zone with higher levels of CA and ST and containing mast cells is located nearby this strip. This is explained by mechanical injury to tissues, inflicted by the needle, and mast cell degranulation, which it promotes.

Spectrofluorometric analysis of bioamines 15 min after 2-min acupuncture in the LI11 AP showed increased concentrations of CA and ST in the epithelium and mast cells at the interface between the reticular layer and hypoderma (Fig. 1). One hour after removal of the needles, the content of CA and ST in the distal LI11 AP reduced: by 50% in mast cells of the deep reticular layer, by 36% in the epithelium, and by 27% in the papillary and reticular layers of the derma. The increase in monoamine levels was less pronounced in the epithelium, dermal papillary layer, and hypoderma of the control zone in comparison with AP. Presumably, this was due to initially lower count of mast cells in the subepithelial area in comparison with their count in AP. Later the fluorescence of skin structures in the control zone (in contrast to AP) returned to the level approximating the initial one.

Before acupuncture, all skin structures had low values of serotonin index (SI): from 1.3 to 2.5, depending on AP location and the studied structure. The lowest SI was detected in the elastic fibers of the reticular layer (2.0) and in mast cells of the distal LI11 AP dermal reticular layer (1.81) (Table 1). The index for the same structures of GV14 AP

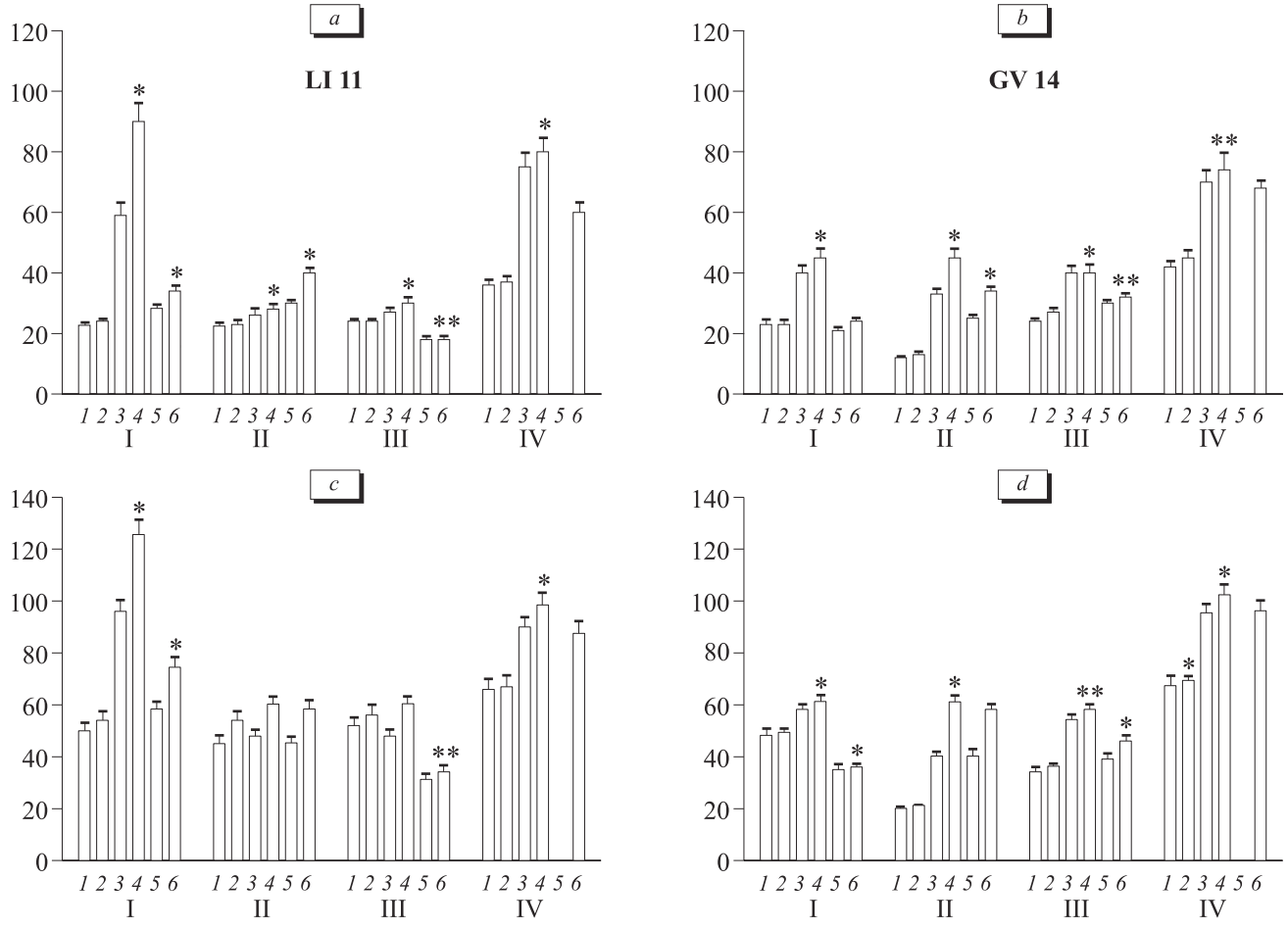

Fig. 1. Content of CA (*a*, *b*) and ST (*c*, *d*) in skin structures of AP after 10-min acupuncture.

TABLE 1. Serotonin Index Values for Skin Structures in LI11 and GV14 AP before Acupuncture (AcP), 15 min and 1 h after 2- and 10-min AcP exposure (*M*±*m*)

AP; skin structure		2 min			10 min		
		before AcP	after AcP	1 h after AcP	before AcP	after AcP	1 h after AcP
LI11	epithelium	2.25±0.12	1.47±0.11	1.18±0.10	2.25±0.14	1.38±0.09	2.17±0.20
	papillary layer fibroblasts	2.36±0.20	1.48±0.11	1.62±0.10	2.36±0.19	1.5±0.1	1.45±0.19
	reticular layer elastic fibers	2.00±0.12	1.39±0.11	1.47±0.12	2.00±0.09	1.44±0.03	1.31±0.07
	hypoderma	2.30±0.09	1.45±0.08	1.48±0.11	2.30±0.09	1.42±0.10	1.37±0.11
	reticular layer mast cells	1.81±0.05	1.48±0.09	1.45±0.10	1.81±0.07	1.22±0.06	1.45±0.09
GV14	epithelium	2.13±0.10	1.57±0.20	1.19±0.11	2.13±0.10	1.35±0.09	1.50±0.07
	papillary layer fibroblasts	1.34±0.11	1.36±0.11	1.37±0.12	1.34±0.11	1.44±0.12	1.32±0.11
	reticular layer elastic fibers	1.79±0.09	1.26±0.08	1.27±0.06	1.79±0.03	1.62±0.08	1.28±0.02
	hypoderma	1.61±0.09	1.33±0.08	1.41±0.07	1.61±0.04	1.35±0.10	1.70±0.09
	reticular layer mast cells	1.53±0.09	1.17±0.09	1.27±0.07	1.53±0.10	1.37±0.06	1.41±0.02

was lower: 1.79 and 1.53, respectively. The highest SI was observed in the epithelium; the mean values were noted for elastic fibers of the reticular layer of both AP. It is known that in rats SI depends on the season [6]. Acupuncture in the studied AP altered the ST-CA proportion. Fifteen minutes after 2-min acupuncture in the LI11 AP the SI in the epithelium reduced to 1.47, in the papillary layer fibroblasts it dropped from 2.36 to 1.47, in elastic fibers of the reticular layer from 2 to 1.39, in the hypoderma from 2.34 to 1.48, and in mast cells from 1.81 to 1.4. The SI changed negligibly 1 h after acupuncture. These data indicate significant redistribution of monoamines in the skin structures, which can be characterized as the initiation of the acupuncture reaction.

Fifteen minutes after removal of the needle the content of CA and ST in GV14 AP increased in the epithelium, mast cells of the dermal deep reticular layer, hypoderma, and remained unchanged in the fibroblasts and mast cells of the dermal papillary layer (Fig. 1). In contrast to the LI11 AP, the content of CA and ST in the epithelium was still increasing 1 h after the procedure (1.47 times). The concentrations of CA and ST in the derma and large mast cells decreased similarly to those in the distal point. In the control zone acupuncture induced a less pronounced increase in the concentrations of CA and ST in the studied structures than in AP, while in the dermal mast cells the content of CA and ST increased 2-fold, which was however lower than their concentrations in AP. Directly after acupuncture SI decreased from 2.13 to 1.57 in the epithelium, from 1.79 to 1.26 in elastic fibers, from 1.61 to 1.33 in the hypoderma, and from 1.53 to 1.17 in mast cells. In contrast to the distal point, SI for the reticular layer fibroblasts virtually did not change. One hour after acupuncture, SI for the derma and mast cells remained unchanged (similarly as in the distal AP), while in the epithelium it reduced from 1.57 to 1.19.

Fifteen minutes after 10-min acupuncture, the content of CA and ST in LI11 AP increased mainly in the epithelium. One hour after the procedure, the content of bioamines decreased by 65% in the epithelium and by 20% in the papillary layer fibroblasts and reticular layer elastic fibers, while in the hypoderma it increased by 48%. Directly after acupuncture, SI decreased in all studied structures and after 1 h it was still decreasing in the papillary layer fibroblasts, dermal reticular layer, and hypoderma. In the control zone, prolongation of acupuncture exposure induced an increase in the content of monoamines in the epithelium and mast cells (less pronounced than in AP), while in the dermal papillary layer and reticular layer elastic fibers and hypoderma prolongation of the procedure did not lead to increase in the monoamine concentrations, similarly as in AP.

Hence, the content of CA and ST increased significantly in the epithelium and mast cells of the dermal deep layer in LI11 AP early after 2- and 10-min acupuncture. After 1 h, the amine concentrations returned to the initial level only in the dermal papillary and reticular layers and in the hypoderma of LI11 AP, while in mast cells of the dermal deep layer it remained high. Mast cells in the subepithelial, perivascular, and perifollicular zones are characterized by low reactivity in comparison with the cells in the dermal deep layer, which manifes-

ted in less pronounced changes in the bioamine concentration. Presumably, this was due to lesser maturity of heparin in them.

After 10-min acupuncture, the levels of CA and ST in GV14 AP increased most markedly in the epithelium, mast cells of the dermal deep layer, and hypoderma, and least of all in the derma. After 1 h, similarly as in LI11 AP, the levels of CA and ST decreased 1.8 times in the epithelium and 1.2-1.3 times in mast cells of the dermal deep layer and in the rest structures. Serotonin index directly after acupuncture and 1 h after it changed similarly to that in the distal AP.

Hence, despite a certain variety of fluctuations in the SI in skin structures in response to acupuncture, the index never dropped below 1 arb. unit. The greatest reduction of SI was noted for fibroblasts of the dermal papillary layer and hypoderma of LI11 AP. Presumably, SI reduction in this or that skin structure after acupuncture was caused by elimination of ST from this structure. It seems that skin structures depositing ST deliver it to AP microzone at different time.

It is noteworthy that not a single fluorescent mast cell was detected 1 h after 10-min acupuncture, which was presumably caused by massive degranulation of these cells. One hour after acupuncture the levels of the studied monoamines in zones other than AP reduced to the initial levels in all the studied structures.

Hence, changes in the bioamine status of the skin start as early as 15 min after acupuncture, but the results of exposure are different for GV14 and LI11 AP: skin structures in the LI11 AP react sooner and more intensely. In the GV14 point, the content of CA and ST is still increasing in the epithelium and hypoderma after removal of the needle. Prolongation of exposure leads to an increase in the content of CA and ST in skin structures, particularly in the LI11 AP. These changes are leveled in various AP after 24 h, and the morphology returns to the initial picture.

REFERENCES

1. V. G. Vogralik and V. M. Vogralik, *Fundamentals of Traditional Eastern Reflex Diagnosis and Puncture Adaptation Therapy* [in Russian], Moscow (2001).
2. D. S. Gordon, V. E. Sergeeva, and I. G. Zelenova, *Lymphoid Organ Neuromediators* [in Russian], Leningrad (1982).
3. V. A. Kozlov, *Localization and Status of Tissue Neurotransmitter Systems in Health and Experiment* [in Russian], Moscow (2006).
4. E. M. Krokhina, L. M. Chuvil'skaya, and E. B. Novikova, *Arkh. Anat., Gistol., Embriol.*, No. 3, 59-61 (1980).
5. L. A. Lubovtseva, *Fluorescent Histochemical Study of Amino-Containing Structures of the Bone Marrow, Thymus, and Blood under the Effects of Neuromediators and Antigens* [in Russian], Cheboksary (1993).
6. S. A. Yastrebova and V. E. Sergeeva, *Mechanisms of Hydrocortisone Immunomodulation of the Thymus cellular Bioamine System* [in Russian], Cheboksary (2000).
7. B. Falck, N. A. Hillarp, G. Thieme, and A. Torp, *J. Histochem. Cytochem.*, **10**, 348-354 (1962).