Analysis of the Effect of Acupuncture on the Regeneration of Rat Submaxillary Salivary Gland using the Clusterization Method

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Cluster analysis of the effect of acupuncture on the regeneration of rat salivary gland reveals a diverse reaction: regeneration is enhanced in 19% of the animals, while in 32% it is suppressed, the intact contralateral part of the gland being atrophied.

Key Words: *salivary gland; acupuncture stimulus; regeneration; cluster analysis*

The salivary gland has a limited ability to regenerate after trauma and responds weakly to pharmacological stimulation [7]. It has been postulated that posttraumatic regeneration of the salivary gland or any other tissue is strictly programmed [3].

Our objective was to study posttraumatic regeneration of submaxillary salivary gland (SMSG) upon stimulation of acupuncture points (AP) on the skin. Acupuncture is known to modulate regeneration [4] and diseases [6] of the salivary glands.

MATERIALS AND METHODS

Experiments were performed on 119 pubertal outbred male albino rats weighing 150-200 g. Right sialotomy of the lower pole of SMSG was performed in all animals under combined anesthesia (75 mg/kg Hexenal intraperitoneally and 1% Novocain locally). Hemorrhage was stopped by swabbing. The wound was closed by layers using silk sutures. Operated animals were divided into three groups. Group I (control) consisted of rats without any postoperative manipulations. In group II rats (placebo - pain and tissue integrity control) [5], ten points (not AP, but so-called inactive AP) on the tail were cauterized. In group III animals, AP were symmetrically cauterized on postoperative days 1, 3, and 5. It was assumed that anatomical localization of AP in rats is the same as in human beings. The points were designated according to French terminology: G1, E36, F2, PN25, and PN60. The rats were euthanized seven days later by Hexenal overdosage. The operated and contralateral SMSG, thymus, and spleen were excised for investigation. The material was dried at 110°C to a constant weight. The following biometric parameters were evaluated: thymus and spleen indices (ratio between organ and body dry weights). In order to exclude uncontrollable factors from the experiment, the regenerated mass of the gland (RMG) was calculated from a covariant analysis formula: $RMG=MOG-0.15\times P_1+3-MEP-0.15\times P_2-P_1/TMG\times 100\%$, where MOG is the mass of the operated gland (mg), P_1 is the body weight prior to the operation (g), P_2 is the body weight before sacrifice (g), MEP is the mass of the excised part of SMSG (mg), and TMG is the "theoretical" mass of SMSG calculated from a linear regression formula with the use of the body mass before sacrifice.

Changes in the mass of the contralateral gland (hypo- or hypertrophy) were calculated from the following formula and expressed as a percentage: hypertrophy of contralateral gland (HCG)=body mass before sacrifice/"theoretical" mass×100%. Data were assessed using cluster analysis [1]. Classification of

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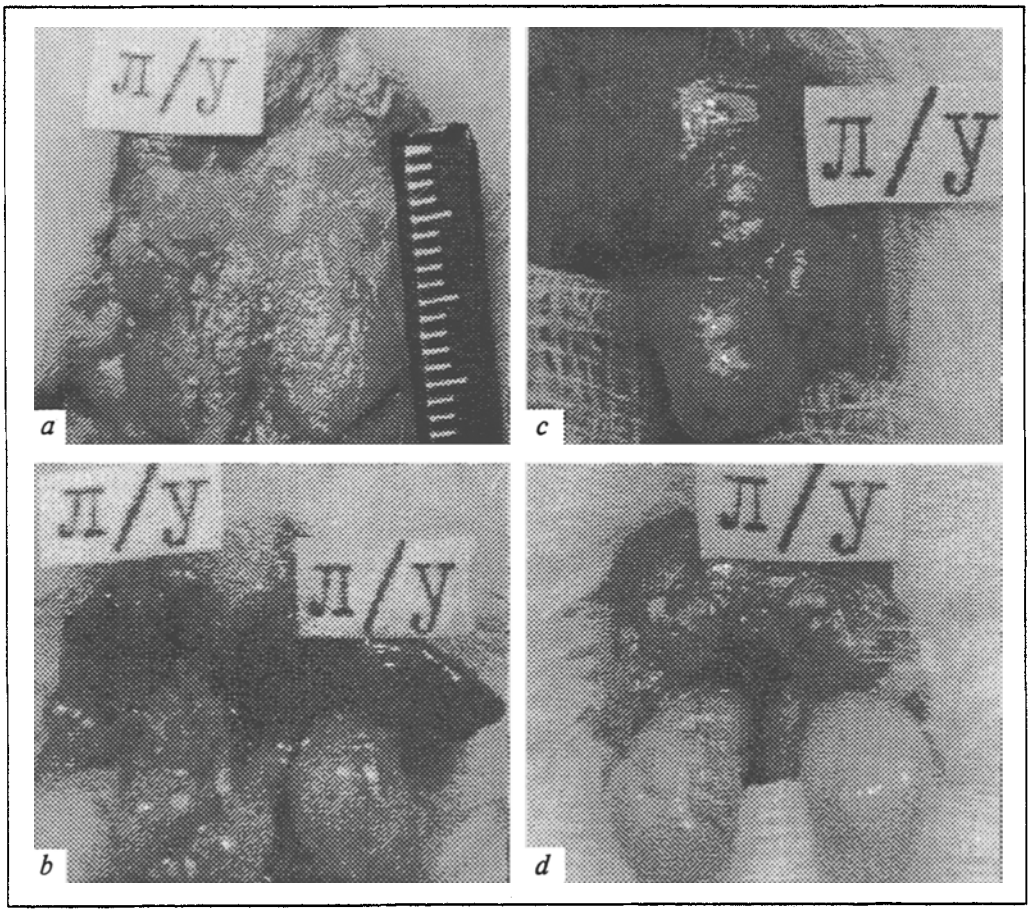

Fig. 1. Salivary glands and regional lymph nodes in intact rats and one week after sialotomy. *a)* SMSG and regional lymph nodes (Л,У) – (*l/n*) of intact rat; *b–d)* operated (*O*) and contralateral (*CL*) SMSG with their lymph nodes one week after sialotomy (cauterization of AP).

groups was performed in a four-dimensional criterion space. The criteria were given the following coefficients of significance: the first criterion (RMG) 1; the second criterion (HCG) 1; the third criterion (thymus index) 0.5; and the fourth criterion (spleen index) 0.75.

RESULTS

Postmortem examination showed that only in group I (cauterization of AP) the lymph nodes regional for the salivary gland were markedly increased and hyperemic in 22% of cases (Fig. 1). Therefore, we decided to examine the immune system organs (thymus and spleen).

Comparison of the results according to one criterion showed that 57±5% of excised mass of SMSG is regenerated one week after sialotomy with a simultaneous development of vicarious edema of the contralateral gland (HCG increased by 28±6%). Comparison of these results with those obtained in other groups (placebo and cauterization of AP) according to each criterion revealed no significant changes in mean values. Hence, we decided to compare groups by several criteria, i.e., using multivariate statistical analysis.

Calculations showed that cauterization of inactive AP (placebo) had no appreciable effect on the regeneration of SMSG. When group I ("pure" control) and group II (placebo) were combined, 2% of observations proved not to fit any cluster and were excluded from analysis. Ninety-three percent of observations from groups I and II were combined in one cluster, and subsequent comparison was performed between the total control and experimental group III (Table 1).

At first the data were classified by three clusters: animals with normal, hypo-, and hyperergic regeneration. In the control group most of the animals (92%) could be assigned to one cluster. The second cluster consisted of rats with a hyperergic reaction: regeneration 12% and hypertrophy 76% higher than in the first cluster. The third cluster included rats with a weak response to trauma: the percent of regeneration was 2-fold lower. However, all other parameters were higher in this group. If the sample is classified by 4-5 clusters, the second and third clusters remain the same in terms of both the number of animals and the mean values of some parameters.

The percent distribution of group III animals (cauterization of AP) into three clusters was dif-

TABLE 1. Changes in Mean Values (Cluster Centers) of Some Parameters of the Submaxillary Salivary Gland and the Immune System Organs after Unilateral Sialotomy with Stimulation of Acupuncture Points

Number of clusters	Composition of cluster	Control (n=66)					Experiment (n=53)				
		%	RMG	ECG	TI	SI	%	RMG	ECG	TI	SI
3	1	92	45	120	0.33	0.74	73	28	114	0.31	0.86
	2	5	57	196	0.31	0.68	23	70	144	0.38	0.96
	3	3	24	164	0.57	0.92	4	29	185	0.30	1.15
4	1	59	57	126	0.31	0.74	41	35	128	0.35	0.76
	2	33	23	109	0.36	0.76	32	19	96	0.25	0.98
	3	5	57	196	0.31	0.68	23	70	144	0.38	0.96
	4	3	23	164	0.57	0.92	4	29	185	0.30	1.15
5	1	58	58	126	0.31	0.71	41	35	128	0.35	0.76
	2	33	23	109	0.36	0.76	26	15	98	0.26	0.51
	3	5	57	196	0.31	0.68	23	70	144	0.38	0.96
	4	3	24	164	0.57	0.92	6	39	88	0.20	0.71
	5	1	43	133	0.38	0.63	4	29	185	0.30	1.15

Note. n is the number of measurements, *TI* is the thymus index, and *SI* is the spleen index.

ferent. The largest cluster consisted of animals with RMG 2-fold lower than in the control. The second cluster included animals with maximum regeneration, their number being 19% higher than in the control. The number of rats with a weaker response (the third cluster, n=24) was practically the same as in a similar cluster in the control (n=29). If the data are classified by four or five clusters, the second and third cluster remain unchanged. At the same time, it can be seen that the first cluster is not homogeneous. It contains 32 out of 73% of cases with not only reduced regeneration but also with HCG less than 100%, i.e., not with hypertrophy but with atrophy of the contralateral gland.

This study was an attempt to assess the effect of acupuncture on posttraumatic regeneration of the submaxillary salivary gland. The difficulties lay in the fact that the exact localization of AP in rats is unknown, and the frequency and duration of stimuli were chosen empirically. Nevertheless, evidence (hypertrophy and hyperemia of lymph nodes adjacent to the salivary glands) was obtained confirming the effect of the specific stimuli. Since the response of lymphoid tissue to acupuncture is documented [2,8], it can be assumed that not only was an influence achieved via AP (cauterization of inactive AP induced no response), but it was specific for the salivary glands, since there was no response from the main organs of the immune system (thymus and spleen).

Thus, cauterization of certain AP induces a diverse response. In some animals (19%) the posttraumatic reaction of the SMSG is enhanced, while in others (32%) regeneration and atrophy of the contralateral intact gland occur.

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