EFFECT OF DESYMPATHIZATION ON REPAIR OF THE RESECTED THYROID GLAND IN ADULT RATS

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Despite all the research which has been undertaken to study regeneration problems, there have been few investigations of the regenerative ability of the desympathized thyroid gland (TG) and the results are contradictory [1, 8, 9]. The variability of the results largely depends on the model of desympathization used. Most investigators have used the surgical method [1, 8, 9], but in this case the possibility of injury to parasympathetic fibers cannot be ruled out. Immunologic and chemical methods of selective destruction of the architectonics of sympathetic terminals [7], with preservation of cholinergic endings, are recognized as most effective.

The aim of this investigation was to study the time course of repair processes in the rat TG after resection of two-thirds of the gland, preceded by partial chemical desympathization.

EXPERIMENTAL METHOD

Experiments were carried out on 65 normally developing and partially desympathized male albino rats of mature reproductive age. Desympathization was carried out by subcutaneous injection of a solution of guanethidine (Isobarin, from "Pliva," Yugoslavia) in a dose of 15 mg/kg body weight, during the first 14 days after birth. This program of injections of guanethidine caused death of more than 80% of sympathetic cells in the rats' nervous system [5]. When reaching the age of 12 months some of the animals in the experimental and control groups underwent resection of two-thirds of TG under ether anesthesia, and were sacrificed 3, 5, 7, and 15 days later. After decapitation, blood serum levels of tri-iodothyronine (T_3), thyroxine (T_4) and thyrotrophin (TTH) were determined by means of the Soviet test kits RIO-T_3-PG and RIO-T_4-PG and the commercial kit RIA-mat TSH ("Byk-Mallinckrodt," West Germany). TG was fixed in Bouin's fluid and embedded in paraffin wax. Sections 4-5 μ thick were stained with hematoxylin and eosin, and the stereologic resorption index (SRI), the height of the thyrocytes, and the mean diameter of the follicles were determined [2]. Proliferative activity was assessed on the basis of autoradiographic data with ^{3}H-thymidine [labeling index of the nuclei (LIN)]. For the electron-microscopic investigation fragments of the organs were incubated in glutaraldehyde and osmium tetroxide and embedded in a mixture of Epon and Araldite M resins. Ultrathin sections were stained with uranyl acetate and lead citrate and extamined in the EVM-100AK electron microscope. The results were subjected to statistical analysis by Student's test for differences between arithmetic means.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the unresected TG of the desympathized rats was in a state of functional stress, as shown by an increase in height of the thyrocytes and a decrease in diameter of the follicles. Electron microscopy revealed dilatation of the cisterns of the endoplasmic reticulum, invagination of the nuclear membrane, and an increase in the relative density of mitochondria and ribosomes in the cytoplasm of the gland cells. A high degree of incorporation of the labeled precursor of DNA synthesis into the thyrocyte nuclei was clearly visible (Table 1). The proliferative activity of the thyroid parenchyma is induced by the adenohypophysis through TTH, and is inhibited by sympathetic mediators, acting as cofactor for the antimitotic action of chalones [3]. The increase which we found in LIN in the

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TABLE 1. Morphological Parameters of TG Regeneration in Normally Developing and Partially Desympathized Rats after Resection of Two-Thirds of the Gland

Parameter	Experimental groups	Units	Series without resection	Subtotal thyroidectomy, days before sacrifice: 3	5	7	15
Diameter of follicles	Control	I	61,16±1,3	43,66±0,76*	42,6±0,82*	47,37±0,93*	47,6±1,05*
		II	100	71,39±1,24	69,65±1,34	77,45±1,52	77,83±1,72
	Experiment	I	57,74±1,13	60,01±1,17	53,48±1,53**	52,86±1,28**	56,52±1,45
		II	100	103,9±2,03***	92,62±2,65***	91,55±2,22***	97,89±2,51***
Height of thyrocytes	Control	I	8,03±0,15	11,27±0,18*	12,48±0,14*	10,98±0,16*	10,9±0,15*
		II	100	140,4±2,24	155,4±1,74	136,7±1,99	135,7±1,87
	Experiment	I	9,69±0,14*	6,28±0,13	11,29±0,16**	10,18±0,21	10,77±0,17**
		II	100	64,81±1,34***	116,5±1,65***	105,1±2,17***	111,2±1,75***
SRI	Control	III	1,05±0,33	2,71±0,25*	4,25±0,37*	1,94±0,08*	2,57±0,39*
		II	100	257,1±23,8	404,8±35,2	184,8±7,62	244,8±37,1
	Experiment	III	1,45±0,07	1,01±0,03**	2,06±0,33	1,67±0,1	1,56±0,08
		II	100	95,24±2,86***	142,1±22,8***	115,2±6,9***	107,6±5,52***
LIN	Control	IV	7,88±0,42	30,18±0,9*	19,13±0,67*	16,84±0,72*	11,61±0,66*
		II	100	383,0±11,4	242,8±8,5	213,7±9,14	147,3±8,38
	Experiment	IV	12,2±0,73*	13,23±0,81	17,93±0,65**	15,07±0,58**	9,53±0,56**
		II	100	108,7±6,61	147,3±5,34***	123,8±4,77***	78,31±4,6***

Legend. *) Significance of differences ($p < 0.01$) between control group and series without resection, **$p < 0.05$ between experimental group and unresected series, ***$p < 0.05$ between control group at corresponding time of resection; I) in μ, II) in % of unresected series of corresponding experimental group, III) in $\mu^{-1} \cdot 10^{-1}$, IV) in promille.

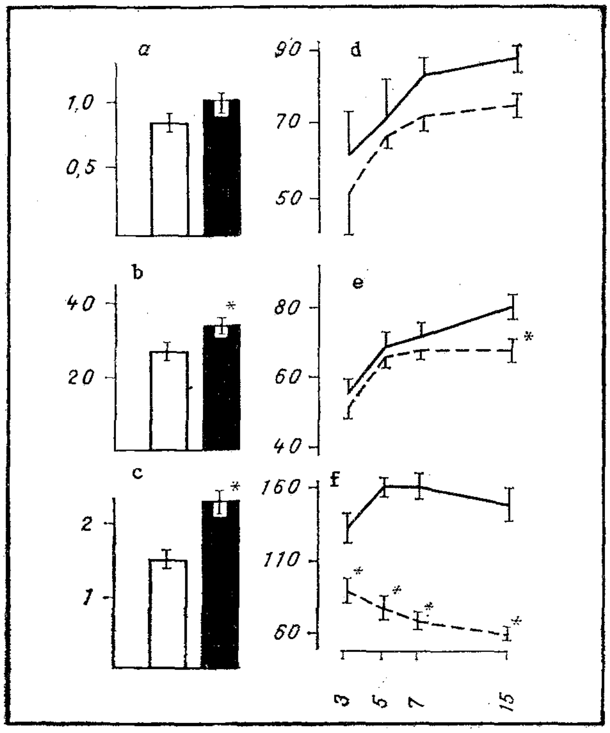

Fig. 1. Dynamics of hormonal profiles of TG: T_3 (a, d), T_4 (b, e), and pituitary TTH (c, f) in rats' blood serum after desympathization (a, b, c) and thyroidectomy (d, e, f). Abscissa: d, e, f) days before sacrifice; ordinate: a, b) mmoles/liter, c) μg/liter, d, e, f) in % of series without resection in corresponding experimental group (continuous line represents control + thyroidectomy; broken line — desympathization + thyroidectomy). Unshaded columns — control animals, series without resection; black columns — desympathized rats without resection. *$p < 0.05$ compared with corresponding parameters of control series.

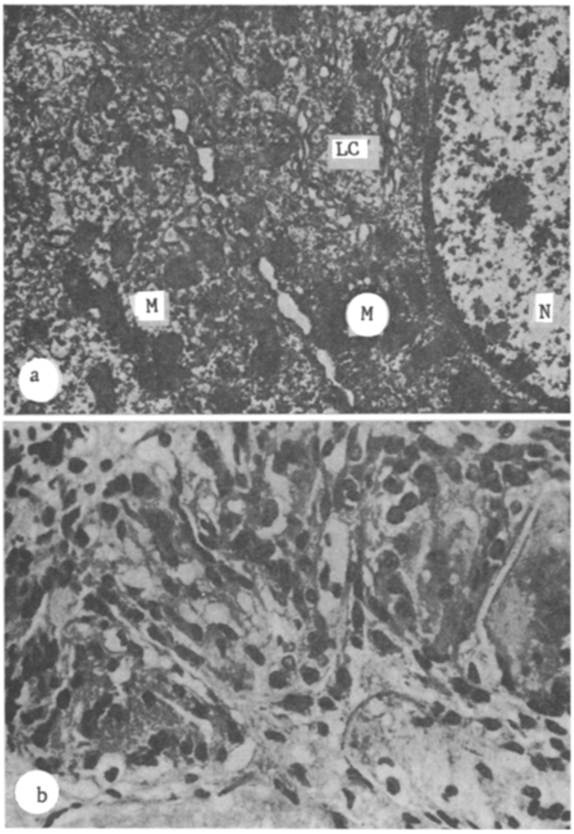

Fig. 2. Structure of TG. a) Consolidation of mitochondria (M) and hyperplasia of vesicles of lamellar complex (LC) in cytoplasm of thyrocyte of control animals on 5th day after operation. 4600×. N) Cell nucleus; b) TG of 12-month-old desympathized rats on 3rd day after thyroidectomy: marked edema of stroma and destruction of follicles with desquamated thyrocytes. Hematoxylin and eosin. 280 ×.

glands of the experimental rats was evidently due to much reduced formation of adrenalin-chalone complexes because of the depressed level of adrenergic mediation and of TTH hyperproduction (Fig. 1c). The more rapid excretion of TTH after denervation has been described by several workers [1, 9]. This phenomenon is regarded as the result of an adaptive reaction of the adenohypophysis in order to restore the specific thyroid function, observed in the early stages after destruction of the adrenergic innervation, and confirmed by the results of our previous investigations [5, 6]. Since the serum T_3 and T_4 concentrations in the experimental rats were higher than the corresponding values in the control group (Fig. 1a, b) it can be tentatively suggested that a new pattern of inter-relations is established after desympathization in animals of mature reproductive age in the hypothalamus – pituitary-TG system, necessitating a higher level of renewal of the strucutral elements, and aimed at strengthening the effector influences of thyroid hormones on target cells. The results are thus evidence of a high degree of structural and functional lability of TG under extremal conditions of function.

Resection of two-thirds of TG in animals with an intact sympathetic nervous system led to a decrease (by 50-40%) in the concentation of thyroid hormones in the blood serum on the 3rd day after the operation (Fig. 1d, e). Reduction of the hormone-forming capacity of the gland led to stimulation of the thyrotrophic function of the pituitary (Fig. 1f). Microscopically, marked structural changes could be detected in the residual gland, with partial destruction of follicles in the zone of the wound surface, the formation of epithelial concentrations, the formation of new adenomeres, and a high level of ^{3}H-thymidine incorporation into thyrocyte nuclei (Table 1). A significant decrease in diameter of the follicles and an increase in the height of the thyrocytes and the value of SRI were recorded, most of all on the 5th day after the operation. At the same times, the serum TTH concentration reached its maximal level (Fig. 1f). Dilatation of the cisterns of the endoplasmic reticulum, hyperplasia of mitochondria, and hypertrophy of the nuclei and vesicles of the lamellar complex were observed in the cytoplasm of the thyrocytes (Fig. 2a). The development of compensatory repair processes in the residual part of the gland led to relative normalization of the thyroid status by the 15th day (Fig. 1d, e), and to some degree this may be connected with hyperproduction of calcitonin by the C-cells of TG during this period [4].

In the desympathized animals, repair processes in TG after subtotal thyroidectomy were characterized by delayed reactivity compared with that of the control animals at the corresponding time after resection. On the 3rd day after the operation, dystrophic changes were observed not only in the region of the wound surface, but also in the central zones of the gland, where consolidation of colloid, flattening and desquamation of the epithelium into the lumen of the adenomere (Fig. 2b), edema and lympho-histiocytic infiltration of the stroma, an increase in diameter of the follicles, and a decrease in SRI were discovered (Table 1). Electron-microscopic investigation revealed vacuolation and shrinking of mitochondria, followed by their fragmentation, rupture of the dictyosomes of the lamellar complex into small, empty vesicles. These changes were local in character. However, repair processes were on a very small scale even at other times after the operation (5, 7, and 15 days; Table 1). Under these circumstances the value of the parameter SRI, reflecting the dynamics of colloid accumulation and excretion, and linked with the functional state of the gland [2], did not differ significantly from that in the group of desympathized animals of the series without resection (Table 1).

Comparison of the relative percentages of the serum T_3 and T_4 levels in the experimental rats with the corresponding parameters of the control animals shows a decrease in the rats of rise of the hormonal profiles of TG after subtotal thyroidectomy in desympathized rats (Fig. 1d, e). The lowered reactivity of the gland was evidently due to a progressive fall in excretion of endogenous TTH into the bloodstream (Fig. 1f). The TTH concentration in the adenohypophysis of desympathized male rats is known to fall after thyroidectomy by 10-30% [1]. Hence it follows that weakening of the thyrotrophic function of the pituitary is connected not only with reduced excretion of the hormonal product into the bloodstream but also, to some degree, with a decrease in its formation. Evidently in long-existing functional stress following desympathization, the additional extremal influence of thyroidectomy causes exhaustion of the compensatory powers of the adenohypophysis, expressed as a disturbance of integrative connections in the hypothalamus—pituitary—TG system and slowing of the rate of normalization of the thyroid status.

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