ACTION OF METHYLCOBALAMINE ON POST-TRAUMATIC REGENERATION OF THE SALIVARY GLANDS

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The course of post-traumatic regeneration of glandular organs depends not only on hormonal factors, but also on the state of the autonomic innervation. However, an important role in the regulation of regenerative processes is played by folate-dependent enzymes, whose activity undergoes considerable changes during disturbances of nerve cell formation in ontogeny, in various neurotoxicoses, and if the diet is deficient in folic acid [6, 8].

Since cobalamine derivatives are known to affect the production of folate-dependent enzymes in cells [7] it was decided to study whether methylcobalamine affects the content of biogenic amines, the lactate dehydrogenase (LDH) isozyme spectrum, and the level of polarization of the secretory cells of the salivary glands after partial resection of the gland tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 109 noninbred rats of both sexes weighing 70-100 g, divided into four groups: 1) animals undergoing resection of one-third of the tissue of submandibular salivary glands 24 h before the experiments; 2) intact animals receiving methylcobalamine in a dose of 500 μg/kg subcutaneously, daily for 3 days, i.e., in doses comparable with therapeutic doses for cyancobalamin in various human diseases [2]; 3) animals receiving methylcobalamine for 3 days, followed by resection of one-third of the tissue of the submandibular salivary gland 24 h before the experiments; 4) intact animals.

The operations and acute experiments on the animals were performed under pentobarbital anesthesia (40 μg/kg, intraperitoneally).

There were two series of experiments. In series I the submandibular salivary glands were dissected in the anesthetized animals and tissue extracts were prepared from them with perchloric acid, homogenized, and analyzed for their content of adrenalin and noradrenalin, in Men'shikov's modification [3]. To determine the LDH isozyme spectrum the gland tissue was homogenized in phosphate buffer and the isozymes determined by electrophoresis [5]. In the experiments of series II the left submandibular salivary gland was isolated in rats and an immobilizing plate made from transparent plastic was introduced beneath it so that the gland did not move when the microelectrode was inserted into its tissue. The temperature of the gland was maintained between 37 and 38°C by irrigation with warm Ringer–Locke solution. The resting membrane potential (MP) was studied by the standard microelectrode technique. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that injection of methylcobalamine for 3 days had no significant effect on the level of polarization of the duct and acinar cells. After trauma to the salivary gland, no changes in the level of polarization of the cells could be found in zones located 4-5 mm from the resected margin. In rats receiving methylcobalamine for 3 days before resection of the salivary glands an increase in the level of polarization of both types of secretory cells of the salivary glands was observed. This is evidence that cells of traumatized salivary glands evidently possess increased sensitivity to methylcobalamine, and its

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TABLE 1. Number of MPs (in %) of Different Types of Submandibular Salivary Gland Cells after Resection of One-Third of Gland and Resection Preceded by Methylcobalamine Injection

MP range, mV	Intact gland (1)	Resection of one-third of gland (2)	Injection of methylcobalamine	Resection of one-third of gland preceded by injection of methylcobalamine (3)
6—10	8,4	5,9	7,5	3,1 ($P_{1-3}<0,05$)
11—15	11,8	9,0	12,3	7,8
16—20	20,8	17,8	22,9	10,9($P_{1-3}<0,01$; $P_{2-3}<0,05$)
21—25	16,3	20,9	16,1	15,3
26—30	14,1	17,0	14,4	17,7
31—35	9,5	9,1	6,8	14,0
Total % of cells of 1st group	80,9	79,7	80,0	68,8 ($P_{1-3}<0,01$; $P_{2-3}<0,01$)
36—40	5,7	5,6	7,2	12,0($P_{1-3}<0,01$; $P_{2-3}<0,001$)
41—45	4,2	4,7	4,7	5,7
46—50	3,4	2,8	4,7	3,1
51—55	3,0	2,4	1,7	3,6
56—60	1,9	1,6	1,3	4,2
61—65	0,4	1,6	0,4	2,1
66—70	0,5	1,2	—	0,5
71—75	—	0,4	—	—
Total % of cells of 2nd group	19,1	20,3	20,0	31,2 ($P_{1-3}<0,01$, $P_{2-3}<0,01$)
Total number of cells recorded	263 ($n=5$)	253 ($n=5$)	236 ($n=5$)	200 ($n=4$)

Legend. Here and in Table 2: n) number of animals.

TABLE 2. LDH Isozymes (in %) and Catecholamine Content (in μg/100 g wet weight of tissue) in Submandibular Salivary Gland Tissue after Resection of One-Third of Gland and Resection Preceded by Methylcobalamine Injection (M ± m)

Parameter studied	Intact gland (1)	Resection of one-third of gland (2)	Injection of methylcobalamine (3)	Resection of one-third of gland preceded by injection of methylcobalamine (4)
LDH isozymes				
LDH-1 + LDH-2	21,5±1,2 ($n=13$)	18,0±0,58 ($P_{1-2}<0,05$) ($n=13$)	23,5±1,5 ($n=10$)	18,1±0,72 ($P_{1-4}<0,05$) ($n=9$)
LDH-3	15,2±0,72	16,0±0,63	13,7±0,5	15,7±1,03
LDH-4 + LDH-5	63,3±1,7 ($n=13$)	66,0±1,02 ($n=13$)	62,8±1,6 ($n=10$)	66,2±1,10 ($n=9$)
Catecholamines				
Adrenalin	0,65±0,02 ($n=15$)	0,69±0,04 ($n=10$)	0,62±0,07 ($n=10$)	1,0±0,08 ($P_{1-4}<0,01$; $P_{2-4}<0,01$) ($n=10$)
Noradrenalin	0,66±0,04 ($n=15$)	0,32±0,04 ($P_{1-2}<0,001$) ($n=10$)	0,83±0,08 ($P_{1-3}<0,05$) ($n=10$)	0,68±0,03 ($P_{2-4}<0,001$) ($n=10$)

pharmacologic effect is probably not merely activation of protein synthesis in the cells, but also potentiation of the activity of the ionic pumps that determine the level of cell polarization.

On the basis of data on changes in mediator and carbohydrate metabolism in traumatized glandular organs and their effects on regeneration [1, 4], the next step was to study changes in the catecholamine content and LDH isozyme spectrum under the same experimental conditions as in the experiments described above.

The data in Table 2 show that resection of one-third of the submandibular salivary gland was accompanied by some decrease in the content of aerobic fractions. Injection of methylcobalamine for 3 days into intact animals caused no changes in the isozyme spectrum, but when methylcobalamine was injected after trauma to the gland the LDH spectrum was maintained on the same level as after partial resection of the gland.

The noradrenalin content in the animals 1 day after trauma to the submandibular salivary gland was reduced by about half, whereas the adrenalin level was virtually unchanged (Table 2). Injection of methylcobalamine into intact animals caused no significant changes in the content of adrenalin and noradrenalin. However, when methylcobalamine was injected after trauma to the gland, an increase in the adrenalin concentration and normalization of the noradrenalin concentration were observed.

An artificial increase in the methylcobalamine level in intact animals thus has no appreciable effect on the level of polarization of the duct and acinar cells or on concentrations of catecholamines and LDH isozymes in the glandular tissue. After partial resection of the submandibular salivary glands, the sensitivity of the gland to methylcobalamine increases, and this coenzyme begins to potentiate activity of the sodium–potassium pump appreciably, thereby raising the level of polarization of the acinar and duct cells. Furthermore, the catecholamine concentrations revert toward normal against this background, despite persistance of some changes in the LDH isozyme spectrum. All these facts taken together are evidence that administration of methylcobalamine can give rise to a distinct therapeutic effect after trauma to the salivary glands.

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