EFFECT OF ADRENERGIC RECEPTOR BLOCKING AGENTS ON MITOTIC ACTIVITY OF THE REGENERATING LIVER

P. A. Vunder, V. P. Vunder, and T. A. Andronova

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The α-adrenergic blocking drug phentolamine was injected into male rats 1 h before resection of 70% of the liver and again 24 h after the operation. Phentolamine inhibited mitotic activity of the regenerating liver. Two injections of propranolol, a β-adrenergic blocking drug, at the same times caused an increase in mitotic activity. It was concluded that adrenalin, which excites β-adrenergic receptors, may inhibit regeneration. By its action through α-adrenergic receptors, however, adrenalin stimulates this process.

KEY WORDS: *Adrenergic-receptor blocking drugs; regeneration of the liver; mitotic activity.*

The view is widely held that stress inhibits mitotic activity. This effect is ascribed to the adrenalin liberated during stress [1, 3, 4]. Partial hepatectomy (removal of about 70% of the liver) is a very powerful stressor. An operation of this sort must be presumed to cause increased secretion of adrenalin. What is the effect of this hormone, liberated after partial resection of the liver, on the subsequent process of regeneration? The answer to this question could be given by experiments in which the action of adrenalin is blocked.

The object of this investigation was to study the effect of α- and β-adrenergic receptor blockade on mitotic activity of the regenerating liver.

EXPERIMENTAL METHOD

Male albino rats with a mean weight of 200 to 330 g in each experiment were used. Up to 70% of the liver was removed from all the animals. The hepatectomized rats were divided into three groups. Group 1 was the control, i.e., two thirds of the liver was resected and two injections of physiological saline were given. The animals of group 2 received propranolol (Obsidan), a β-adrenergic blocking drug, 1 h before and again 24 h after resection of the liver. The first injection was given in a dose of 12 mg/kg, the second in a dose of 6 mg/kg; the animals of group 3 also received two injections, in this case of the α-receptor blocking drug phentolamine. Both injections were given in a dose of 20 mg/kg. Since the experiments in which phentolamine was given were carried out on animals of different weight from those used in the experiments with propranolol, a special control was included (partial hepatectomy). The animals were decapitated 30 h after partial hepatectomy, which was always performed at 3-4 p.m. Pieces of liver were fixed in Carnoy's fluid and embedded in paraffin wax. Sections 5-6 μ thick were stained by Feulgen's method. The number of mitoses and of the consecutive phases of mitosis were counted in 100 fields of vision of the microscope with a magnification of 40 × 50. The mitotic index and the phase coefficient, i.e., the ratio between the total number of prophases and metaphases and the total number of anaphases and telophases, were calculated. The number of the individual phases of mitosis was ex-

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TABLE 1. Effect of Blockage of α- and β-Adrenergic Receptors on Mitotic Activity of the Regenerating Liver (M ± m)

Group No.	Experimental conditions	Number of animals	Mitotic index	Prophases	Metaphases	Anaphases	Telophases	Phase coefficient
				in 100 fields of vision of the microscope (in %)				
1	Control (partial hepatectomy)	10	6,0±0,95	56,58	21,19	6,38	15,85	3,54
2	Partial hepatectomy + injection of propranolol	10	10,39±1,5 $P_{1,2}<0,05$	56,91	19,51	5,59	17,99	3,24
3	Control (partial hepatectomy)	8	7,98±1,3	61,75	21,1	5,52	11,62	4,83
4	Partial hepatectomy + injection of phentolamine	9	0,85±0,1 $P_{3,4}<0,001$	71,94	12,20	4,88	10,98	5,31

pressed as a percentage of the total number of mitoses. The liver glycogen content and the weight of the adrenals also were determined*.

EXPERIMENTAL RESULTS

As Table 1 shows, two injections of propranolol caused a statistically significant increase in the mitotic index. The change in the phase coefficient was not significant. This suggests that propranolol stimulates mitotic activity. It can accordingly be concluded that under natural conditions adrenalin, which excites β-adrenergic receptors, inhibits mitotic activity of the regenerating liver.

Opposite results were obtained by administration of phentolamine. This blocking agent depressed mitotic activity: The mitotic index was 0.85 ± 0.1 compared with 7.98 ± 1.3 in the control (groups 3 and 4). Meanwhile the percentage of prophases and the phase coefficient were high, although the absolute number of prophases was low. Whereas in the control group the number of prophases was 45.6 ± 8.09, in the group of animals receiving phentolamine it was 6.6 ± 1.07. It can be concluded from the experimental results that adrenalin, by acting through α-adrenergic receptors, stimulates regeneration in the liver.

What is the mechanism of the stimulating effect produced by injection of propranolol? It can tentatively be suggested that β-adrenergic receptor blockade led to inhibition of adenyl cyclase activity. As a result, synthesis of cyclic AMP was reduced, and this led to stimulation of mitosis. However, evidence against this argument is given by the fact that propranolol, if given to rats 18 h after resection of the liver, had no effect on the cyclic AMP content of the organ [8]. In the present experiments propranolol (like phentolamine) did not prevent a fall in the glycogen content in the regenerating liver. This is indirect evidence that the cyclic AMP level in the regenerating liver of rats receiving propranolol was adequate. Moreover, in the writers' opinion, cyclic AMP cannot be regarded as an inhibitor of mitosis in the liver, for shortly before a burst of DNA synthesis in the regenerating liver two waves of increase of the cyclic AMP concentration are observed [6], and there is other evidence [7] of three such waves. Furthermore, injection of adrenalin together with glucagon and theophylline, which sharply increase the cyclic AMP concentration in the liver, not only does not delay normalization of the weight of the liver after resection of 70% of its mass, but actually accelerates this process [2].

The foregoing facts suggest that the stimulating action of propranolol on mitotic activity of the regenerating liver is based on a mechanism unconnected with a reduction of its cyclic AMP content.

These observations, showing the inhibitory effect of phentolamine on mitotic activity in the liver, are in agreement with results obtained by Thrower and Ord [8], who showed that a single injection of phentolamine 18 h after resection of the liver inhibits DNA synthesis. On the basis of these workers' findings it can be postulated that the decrease in mitotic activity observed in the present experiments after two injections of phentolamine was also due to inhibition of DNA synthesis. However, when the inhibitory effect of phentolamine on mitotic activity is interpreted the stressor action which this blocking agent evidently has

*L. G. Perfil'eva also helped with the experiments.

on the body must be taken into account. The fact is that in rats receiving phentolamine the adrenals were found to be increased in weight by 44.9%: They weighed 28.58 ± 1.18 mg % compared with 19.72 ± 1.56 % in the control. Also it was in the rats of this group that the greatest decrease in the glycogen content took place in the regenerating liver. These changes must be interpreted as the result of the stressor action of phentolamine. Accordingly the writers are inclined to conclude that the inhibitory effect of phentolamine on regeneration in the liver is not merely the result of α-adrenergic receptor blockage, i.e., abolition of the stimulating action of adrenalin, but is also to some degree the result of the stressor effect of this blocking agent.

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EFFECT OF REGENERATION OF THE SPLEEN AND BONE MARROW ON NUMBER OF HEMATOPOIETIC COLONIES IN THE MOUSE SPLEEN

G. K. Sukhova, T. R. Podrabinek, and G. V. Kharlova

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Two thirds of the spleen (group 1) or the bone marrow from the right tibia (group 2) was removed from sexually mature male CBA mice. On the eighth day after lethal irradiation and injection of $1 \cdot 10^6$ nucleated cells from the intact spleen the number of hematopoietic splenic colonies was counted. A significant increase in the number of colonies was observed in the animals of both experimental groups compared with the control intact mice. The authors suggest that this increase may have been caused both by the local effect of the regenerating splenic stroma and by a certain stimulating factor secreted by the regenerating hematopoietic tissue.

KEY WORDS: *Regeneration of the spleen; regeneration of bone marrow; hematopoietic splenic colonies.*

The stroma of hematopoietic organs has been shown [7, 10, 12] to be the decisive factor in the choice of the way of differentiation of stem cells, and the number of exogenous colonies formed per spleen in irradiated mice depends entirely on the number of donor cells injected into them. The possibility cannot be ruled out that under certain conditions (regeneration, for example) the stroma may also affect the number of splenic colonies.

Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Department of Histology, Biological Faculty, Moscow State University.

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