INVESTIGATION OF RECEPTORS MEDIATING SEROTONIN STIMULATION OF MOUSE LIVER REGENERATION

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Serotonin stimulates proliferation in the bone marrow [9], growth of fibroblasts in culture [2, 12], and morphogenesis of the sea urchin [13], but lowers the mitotic index (MI) in the cornea [9] and inhibits growth of many tumors [9, 10]. The effect of serotonin on other cell types has not been demonstrated or is disputed [2, 9, 12], and for that reason its role in proliferation is considered to be critical [2, 12, 13] or doubtful [15].

One of the best models with which to study proliferation is the regenerating liver, for the operation causes at least 60% of the hepatocytes to embark upon mitosis [4]. It has been stated that serotonin in this model activates cell division [9], but the authors cited used a very large dose of serotonin — 142 μmoles/kg. The receptor mechanisms of this effect were not studied.

The aim of the present investigation was to discover optimal conditions for the proliferative effect of serotonin on the regenerating liver and to analyze the role of serotonin receptors in this effect.

EXPERIMENTAL METHOD

Experiments were carried out on 200 female (CBA × C57BL)F_1 mice aged 2-3 months. Partial resection of the liver was performed by the usual method of Higgins and Anderson in the evening (8-9 p.m.) and the animals were killed 36 h later (at 8-9 a.m.). The degree of proliferation was judged from changes in MI. Material was fixed in Carnoy's mixture and sections 5-6 μ thick were stained with hematoxylin and eosin. No fewer than 5000 cells were counted in each preparation. Serotonin (5.7 μmoles/kg) and its agonists — mexamine (5-methoxytryptamine, 53 μmoles/kg) and α-naphthylbiguanide (4.4 μmoles/kg) were injected subcutaneously 4 h after the operation. Lysenyl* (0.29 and 2.9 μmoles/kg) and morphine (6.9 μmoles/kg) were injected subcutaneously 20 min before serotonin; tipindole (3.3 μmoles/kg) was injected intraperitoneally 5 min before serotonin. The doses used and the times of administration of the drugs were optimal [8]. The serotonin concentration in the liver was determined by the method described in [5].

EXPERIMENTAL RESULTS

Serotonin in a dose of 5.7 μmoles/kg considerably increased MI of the hepatocytes (Fig. 1). With an increase in dose of the amine by an order of magnitude, the effect disappeared. The small dose of serotonin which was used can be regarded as physiological, for it corresponds to the concentration of this amine in normal liver (7.7 μM). Data indicating the opposite action (inhibitory) of serotonin on proliferation may perhaps be due to the use of two large doses. The optimal time of administration of serotonin was 6 h after the operation which corresponds to the G_0 phase of the cell cycle [4]. After 12 and 18 h the effect decreased and ceased to be significant. This is in harmony with maximal stimulation of cell growth in culture after addition of serotonin at the time of feeding [12].

*Alternative name Lisuride.

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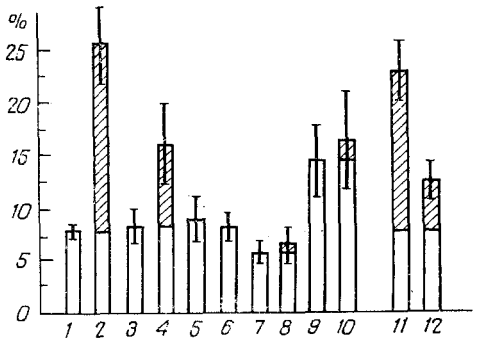

Fig. 1. Effect of serotonin and its agonists and antagonists on mitotic activity in the regenerating liver. 1) 0.9% NaCl solution; 2) serotonin creatinine-sulfate 5.7 μmoles/kg; 3) lysenyl 0.29 μmoles/kg; 4) the same + serotonin; 5) lysenyl 2.9 μmoles/kg; 6) the same + serotonin; 7) morphine 6.9 μmoles/kg; 8) the same + serotonin; 9) tipindole 3.3 μmoles/kg; 10) the same + serotonin; 11) mexamine 53 μmoles/kg; 12) α-naphthylbiguanide 4.4 μmoles/kg. Ordinate, MI (in %).

Consequently, serotonin may be an early stimulus facilitating the entry of cells into mitosis. The greatest increase in MI was observed 36 h after the operation (30 h after injection of serotonin). Later these differences from the control with respect to this parameter disappeared, but by the 5th day the weight of the liver in the experimental series was completely back to normal (this was not found in the control even after 8 days; $P < 0.05$). Serotonin evidently stimulates not only proliferation, but also growth of new cells [1].

To study the role of serotonin receptors in regeneration, two classical approaches were used: comparison of the degree of blocking of the serotonin effect by specific blockers and reproduction of the serotonin effect by more specific agonists.

Reproduction of the serotonin effect by mexamine, an agonist with respect to its influence on D receptors — in a dose equieffective to serotonin on other pharmacological D effects [8], is evidence in support of activation of D receptors. This conclusion is supported by the considerable blocking of the action of serotonin by the D antagonist lysenyl in an ordinary dose. Consequently, stimulation of proliferation is more resistant to lysenyl than the ordinary D effects of serotonin.

Complete blocking of the serotonin effect by morphine in an ordinary dose is evidence in support of activation of M receptors. This conclusion is not contradicted by the weak effect of α-naphthylbiguanide, for it acts on M receptors only in higher doses [8]. Mexamine, however, with practically no effect on M receptors, stimulated proliferation just as strongly as serotonin. Unlike serotonin, it inhibited growth of fibroblasts in culture [12]. An important role of T receptors seems least likely for the T agonist α-naphthylbiguanide gave only a weak effect. Admittedly, the results with tipindole are evidence in support of its having the properties of a partial agonist-antagonist, as has already been noted previously [6]. However, the action of both tipindole alone and atipindole with serotonin was weaker than that of serotonin itself ($P < 0.1$). On the whole, the serotoninergic structures through which proliferation is stimulated cannot thus be classed in any one "pure" type. The only possible suggestion is that they are undifferentiated in character and can therefore interact with both D- and M-serotoninergic substances (both agonists and antagonists) and also, to a lesser degree, even with T-tropic substances. The blocking action of both D and M antagonists was observed previously for some of the central effects of serotonin [3] and its thiol-enhancing effect [6], on the basis of which it has been suggested that undifferentiated serotonin receptors may exist [6]. The present results are in good agreement with the view that blockers of all three types of serotonin receptors prevent the effect of the amine on growth of fibroblasts in culture [2].

TABLE 1. Effect of Serotoninolytics on Serotonin Accumulation in the Regenerating Liver

Series of experiments	Without blockers	Lysenyl 2.9 μmoles/kg	Morphine 6.9 μmoles/kg	Tipindole 3.3 μmoles/kg
Without serotonin	3,68±0,13	2,29±0,52**	2,40±0,85*	2,55±0,16**
Injection of serotonin	4,34±0,49	3,80±0,58	4,34±0,56	2,84±0,47
Increase	+0,66	+1,51**	+1,94**	+0,29

*P < 0.1, **P < 0.05 (in top row compared with control, in bottom row significance of increase).

The second stage of the work was to determine the serotonin concentration in the regenerating liver in all the experimental series described above. The reason for this step was the view that a decisive role is played in the realization of the antitumor [9] and radioprotective [11] effects of serotonin not by its interaction with receptors, but simply by its concentration in the tissues. The serotonin concentration in the liver was depressed by 52 ± 5% 36 h after the operation. Administration of serotonin at the time of testing (40 h later) did not cause any significant accumulation of the amine in the liver (Table 1). This was due to the gradual metabolism of this amine, for in the earlier stages (2 h after injection) there was considerable accumulation of serotonin (+3.6 ± 0.49 μM, or +98%).

It is important to note that lysenyl and morphine completely abolished the increase in MI under the influence of serotonin but did not prevent (they actually increased) the rise of its concentration in the tissue. Meanwhile morphine and lysenyl lowered the serotonin level but did not affect MI. On the whole correlation was absent between the effect of serotonin on MI and its concentration and accumulation in the tissue (r_s = +0.45; P > 0.1). This is evidence that it is not simply a question of the concentration of serotonin in the tissue but its interaction with receptors. Serotonin accumulation in the tissue can evidently play the role only of a reserve for subsequent mobilization and action on receptors, as has already been discussed in the case of correlation between the serotonin level and radioresistance [14].

Small doses of serotonin, when injected in the early stages after subtotal hepatectomy, thus stimulate regeneration of the liver. This effect is realized through receptors of undifferentiated type and it does not correlate with the accumulation of serotonin in the liver tissue. Since serotonin is a therapeutic substance [7], its effect on proliferation must be taken into account. It would be useful to test small doses of serotonin for the stimulation of proliferation of the liver and other tissues under clinical conditions.

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BINUCLEAR CELLS DURING POSTEMBRYONIC DEVELOPMENT AND REGENERATION OF THE CHICKEN LIVER

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An important feature of postnatal growth and regeneration of the mammalian liver is that an important role in these processes is played by somatic polyploidization, which is accompanied by the appearance of a population of binuclear cells, averaging 17-20% of the total number of cells [3, 7, 11, 15]. The possible mechanism of formation of these cells is considered to be amitotic division of the polyploid nucleus [3, 7, 8]. The number of binuclear cells in the mammalian liver is subject to regular changes in postnatal development during the 24-h period with changes in the functional load on the organ [1, 8, 11]. A considerable (3-10-fold) decrease in the number of binuclear cells has been described in the rat, rabbit, mouse, and monkey liver in the early stages after partial hepatectomy [2, 10, 11]. It has been suggested that binuclear cells are the source of formation of mononuclear tetraploid [10, 15] cells, although there are data [14] to show that there are no significant changes in the number of binuclear and polyploid cells in the regenerating rat liver in different age groups and that regenerative growth of the organ is entirely on account of an increase in the number of cells.

In this investigation changes in the number of binuclear cells were studied over a period of time in the chicken liver during postembryonic development and after partial hepatectomy. This subject has not been studied previously [4, 6, 12].

EXPERIMENTAL METHOD

The intact and regenerating liver of chickens aged 40 days, 5-6 months, and one year or more was studied. Part of the right lobe, accounting for 1/5-1/4 of the weight of the liver, was removed from the experimental birds. Material for investigation was taken from the resected lobe and from the left, intact lobe. Pieces of liver were fixed in Bouin's and Carnoy's fluids and processed by the usual histological methods. All experimental and control birds were killed at the same time of day.

EXPERIMENTAL RESULTS

Binuclear cells are rarely found in the intact chicken liver at the age of 40 days and they account on average for not more than 0.3% of the total number of cells (Table 1).

The number of binuclear cells in both lobes one day after resection of the liver in chickens aged 40 days was increased more than threefold. Later their number continued to rise, to reach a maximum 15-30 days after the operation, when it was 5-6 times higher than the control. The number of binuclear cells in the liver two months after hepatectomy was

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