PHYSIOLOGY

Role of S2 Receptors in the Stimulatory Effect of Serotonin on Hemopoietic Bone Marrow Stem Cells

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Studies by the method of splenic exocolonies with serotonin receptor blockers methysergide and cyproheptadine showed that S2 receptors are involved in the stimulatory effect of serotonin on hemopoietic bone marrow stem cells.

Key Words: *serotonin; hemopoietic stem cells; receptors*

Serotonin regulates various functions and biochemical processes in organisms. This compound modulates functional activity of the hemopoietic system. Our previous studies showed that serotonin increases the count and stimulates proliferative activity of hemopoietic bone marrow stem cells forming colonies in the spleen (CFUs) of lethally irradiated mice [2]. These data suggest that the effects of serotonin on the hemopoietic tissue are realized via a receptor mechanism. There are at least 3 types of serotonin receptors (S1, S2, and S3 receptors). Activation of these receptors produces different physiological effects [4,5].

Here we studied the role of S2 receptors in the stimulatory effect of serotonin on hemopoietic bone marrow stem cells.

MATERIALS AND METHODS

Experiments were performed on 3-4 month-old CBA mice weighing 20-25 g. The count of colony-forming cells in the bone marrow was estimated by the method of splenic exocolonies [6]. Donor mice were subcutaneously injected with serotonin creatinine sulfate (50 mg/kg, Sigma). S2 receptor blockers methysergide (0.1 and 0.5 mg/kg, Serva) and cyproheptadine (10 and 20 mg/kg, Sigma) were injected intraperitoneally 4 h before isolation of bone marrow cells [2]. Bone marrow cells (5×10^4 in 0.5 ml) were injected into the caudal vein of γ-irradiated recipient mice (7.5 Gy, RUM-17 device, 3-4 h before treatment). Splenic colonies fixed in Bouin fluid were counted 9 days after cell transplantation.

The intensity of proliferation was estimated by the number of colonies in recipients after transplantation of bone marrow cells preincubated with 10^{-6} M cytosine arabinoside (CAB, Upjohn) at 37°C for 1 h. The percent of proliferating CFUs (S-phase cells) was calculated by the formula:

$$\frac{\text{A-B}}{\text{A}} \times 100\%,$$

where A and B are the numbers of colonies without and after treatment, respectively.

The results were analyzed by Student's *t* test [1].

RESULTS

Methysergide and cyproheptadine dose-dependently decreased the count of CFUs in the bone marrow. Methysergide produced a more potent effect compared to cyproheptadine (Fig. 1).

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It was shown that the effects of methysergide and cyproheptadine are realized via blockade of S2 receptor. However, these blockers also display affinity for receptors of other biologically active substances, including histamine, dopamine, acetylcholine, and epinephrine. These compounds act as serotonin receptor agonists or antagonists depending on the type of receptors [3].

We studied whether the effects of these blockers on hemopoietic cells are associated with the influence of serotonin. Serotonin was injected 20 min after administration of blockers to donor mice. Methysergide and cyproheptadine were used in doses producing the most drastic decrease in the count of CFUs (0.5 and 20 mg/kg, respectively). Serotonin abolished the inhibitory effect of antagonists on hemopoietic stem cells. The count of CFUs in these animals returned to the control (Fig. 2).

We also studied the effects of serotonin and its antagonists on proliferative activity of CFUs to determine the cause of changes in the number of colonies produced by these compounds (Table 1).

In normal bone marrow CAB eliminated 11% S-phase CFUs. Four hours after injection of serotonin the content of S-phase CFUs increased to 39%. Methysergide and cyproheptadine decreased the count of proliferating cells to 16 and 15%, respectively. However, after combined treatment with serotonin and these blockers the ratio of S-phase CFUs was 34 and 28%, respectively.

Our results indicate that the effects of serotonin on hemopoietic bone marrow stem cells are realized via a receptor mechanism. Therefore, this biogenic amine can be considered as an endogenous physiological regulator of hemopoiesis.

TABLE 1. Effects of Serotonin and Serotonin Receptor Blockers on Proliferation of CFUs in the Bone Marrow of Donor Mice (*M*±*m*, *n*=8-11)

Experimental conditions	CFUs per 5×10^4 bone marrow cells	
	without CAB	with CAB
Control	12.8±1.2	11.40±1.35
Serotonin	23.50±1.34	14.20±0.86*
Methysergide	8.65±0.38	7.24±1.15
+serotonin	15.80±1.08	10.2±0.9**
Cyproheptadine	8.22±1.00	7.55±0.50
+serotonin	14.88±1.10	10.70±1.24**

Note. *$p<0.001$ and **$p<0.05$ compared to mice without CAB.

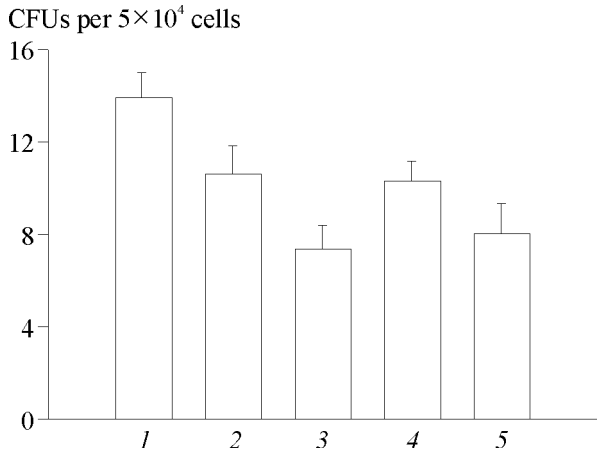

Fig. 1. Effects of serotonin receptor blockers on the formation of splenic CFU in the bone marrow of donor mice: control (*1*), methysergide in doses of 0.1 (*2*) and 0.5 mg/kg (*3*), and cyproheptadine in doses of 10 (*4*) and 20 mg/kg (*5*).

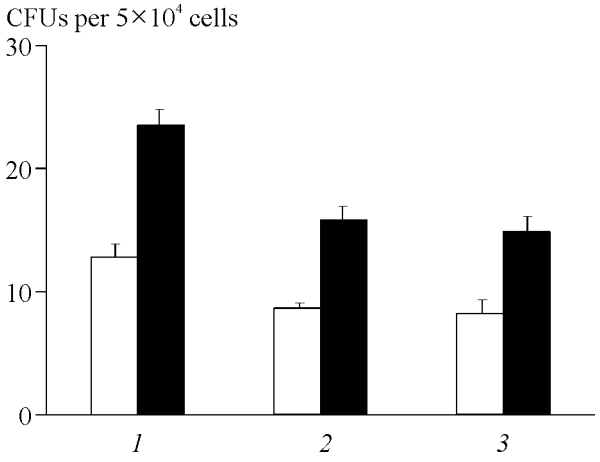

Fig. 2. Effects of serotonin, methysergide, and cyproheptadine on the count of splenic CFU (CFUs) in the bone marrow of mice: control (*1*), methysergide (*2*), and cyproheptadine (*3*). Light bars: without serotonin. Dark bars: in the presence of serotonin.

REFERENCES

1. G. F. Lakin, *Biometry* [in Russian], Moscow (1980).
2. V. P. Nefedov, V. V. Nefedova, V. S. Kononykhina, and I. I. Morgulis, *Byull. Eksp. Biol. Med.*, **112**, No. 11, 488-489 (1991).
3. V. P. Sergeev and N. L. Shimanovskii, *Receptors for Physiologically Active Substances* [in Russian], Moscow (1987).
4. S. J. Peroutra, *Trends Neurol. Sci.*, **11**, 496-504 (1988).
5. A. W. Schmidt and S. J. Peroutra, *FASEB J.*, **3**, 2242-2249 (1989).
6. J. E. Till and E. A. McCulloch, *Radiat. Res.*, **14**, 213-222 (1961).