Activation of platelets, manifested as the formation of pseudopodia, causes activation of the serotonin system: an increase in specific binding of imipramine and of SRU. During the formation of pseudopodia, the platelet membrane becomes deformed, stretched, and it can be tentatively suggested that as a result of this process changes take place in the conformation of the receptor complex, and/or in its accessibility for binding with the ligand, and this in turn brings about activation of the serotonin system.

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EFFECT OF SEROTONIN ON HEMATOPOIETIC STEM CELLS IN BONE MARROW

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KEY WORDS: serotonin; hematopoietic stem cell; bone marrow; proliferation

Serotonin (5-HT), which performs the functions of neurotransmitter and tissue hormone in the body, is involved in the regulation of biochemical and physiological processes under both normal and pathological conditions [4]. The effect of 5-HT on cell proliferation and, in particular, on hematopoiesis is particularly interesting. There is conclusive evidence that exogenous 5-HT has a stimulating effect on erythropoiesis [8, 10] and immunogenesis [1]. High concentrations of endogenous 5-HT have been found in hematopoietic and immunocompetent tissues [2], as well as marked changes in the 5-HT level in these tissues in response to stimulation of erythropoiesis [3]. It has been shown that the amine stimulates cell proliferation in a culture of fibroblasts [5, 7]. It will be noted that the effects of 5-HT on hematopoiesis have been studied as a rule in systems for evaluating the functional activity of mature cells. Taking account of the fact that hematopoiesis is based on the hematopoietic stem cell (HSC), it was decided to study the effect of 5-HT on this particular population of hematopoietic stem cells.

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TABLE 1. Changes in Content and Proliferative Activity of 9- and 12-Day BM CFU_c after Single and Repeated Injections of 5-HT into Donor Mice (M ± m)

Time after injection of 5-HT, h	Femoral karyocytes $\times 10^6$	CFU_c-9 per 5×10^4 BM cells	CFU_c-9 in S-phase, %	CFU_c-12 per 5×10^4 BM cells	CFU_c-12 in S-phase, %
		Single injection of 5-HT			
Control	18,9±0,75 (7)	12,3±0,53 (25)	12	7,85±0,59 (23)	10
1	19,5±1,31 (5)	20,3±0,44* (18)	38	13,8±0,87* (20)	17
3	21,5±1,40 (6)	22,8±1,20* (16)	50	15,2±1,20* (17)	16
6	22,3±1,38 (5)	19,4±0,50* (15)	45	15,0±1,10* (18)	24
24	25,4±2,05 (5)	18,3±0,79* (17)	40	12,8±1,02* (19)	36
48	25,6±1,77 (5)	12,8±1,06 (15)	14	10,0±1,25 (18)	21
		Repeated injections of 5-HT			
Control	19,2±1,30 (6)	13,5±0,90 (20)	14	8,44±0,80 (19)	13
24	27,9±1,05* (5)	10,0±0,71 (19)	47	16,8±0,95* (17)	35

Legend. Asterisk indicates values for which $p < 0.05$. Number of animals given in parentheses.

The aim of this investigation was to study the effect of single and long-term administration of 5-HT on the content and proliferative activity of bone marrow (BM) HSC, forming splenic exocolonies on the 9th and 12th days (CFU_c-9 and CFU_c-12) in lethally irradiated mice.

EXPERIMENTAL METHOD

Female (CBA × C57BL/6)F_1 mice weighing 20-23 g and aged 3-4 months were used. Serotonin-creatinine sulfate ("Sigma," USA) was injected into normal donor mice subcutaneously in a single dose of 50 mg/kg or in repeated doses of 0.5 mg/kg daily for 7 days. Control animals received an injection of 0.2 ml physiological saline.

The BM HSC population was studied by the splenic exocolonies method [12]. For this purpose, at various times after injection of 5-HT, cell suspensions were prepared from the femoral marrow (from 2-3 donors) in Hanks' solution and injected intravenously in a dose of $5 \cdot 10^4$ cells into lethally irradiated (7.5 Gy) recipients (10 mice in a group). Nine and 12 days after transplantation of the cells the number of colonies was counted in spleens fixed beforehand in Bouin's fluid. The proliferative activity of the CFU_c was studied in vitro by the suicide method [13]. BM cells were suspended in α-MEM medium ("Gibco") and $5 \cdot 10^6$ cells in 1 ml were incubated with cytosine arabinoside ("Upjohn") in a concentration of 10^{-3} M for 1 h at 37°C. The intensity of proliferation was judged from the ratio of colony formation in the spleens of irradiated animals after injection of intact hematopoietic cells or of the same cells but after treatment with the cytostatic, which kills actively proliferating cells in the S-phase of the cell cycle. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The BM CFU_c population is known to be heterogeneous: 9-day CFU_c consist mainly of erythroid precursors, whereas 12-day CFU_c consist mainly of polypotent cells [9]. As Table 1 shows, 1, 3, 6, and 24 h after a single (50 mg/kg) injection of 5-HT into donor mice, the number of CFU_c in BM was sharply increased when the number of splenic colonies was recorded on the 9th and 12th days in irradiated recipients. However, the effect of 5-HT was of short duration, for after 48 h the CFU_c level did not differ from that observed initially.

To study the causes of the change in the number of colonies under the influence of 5-HT the proliferative activity of HSC was studied. As Table 1 shows, a single injection of 5-HT in the course of 24 h led to an increase in the number of 9-day CFU_c (38-45%) and of 12-day CFU_c (16-3670) in BM in the S-phase of the cell cycle. Under these circumstances proliferation of CFU_c-12 remained increased 48 h after injection of 5-HT also.

In response to repeated (0.5 mg/kg daily for 7 days) injections of 5-HT the changes in the HSC subpopulations were almost similar in character. Besides activation of proliferation of the two CFU subpopulations in BM 1 day after repeated injections of 5-HT, the total number of karyocytes and also the number of CFU_c-12 were significantly increased. The number of 9-day CFU_c, however, did not differ from that in the control.

It was natural to suggest that stimulation of hematopoiesis in BM is connected with a direct action of 5-HT on HSC. To test this hypothesis, BM cells obtained from normal animals were incubated for 15 min with different concentrations of 5-HT (10^{-5}-10^{-9} M), and were later transplanted into recipients. In concentration of 10^{-8} M 5-HT significantly increased the number of 9-day CFU_c in BM: the number of colonies was 20.4 ± 1.04 in the experiment and 13.4 ± 1.54 in the control ($p < 0.05$).

These results are evidence that 5-HT has a stimulating effect on HSC, forming colonies in the early and late period after irradiation. They also show that the effect of 5-HT may be manifested as triggering of resting CFU_c into proliferation. Considering that the action of 5-HT on erythropoiesis may be mediated through increased production of erythropoietin [8, 10], it can be tentatively suggested that 5-HT stimulates HSC which are committed in the erythroid direction. However, in the case of chronic administration of 5-HT, no increase was found in the number of 9-day CFU_c in BM despite an increase in the number of cells in the S-phase of the cell cycle. It is probable that 5-HT increases the release of CFU_c-9 into differentiation without any increase in their number. The possibility of a direct effect of 5-HT on HSC likewise cannot be ruled out, for it has been shown that 5-HT is contained in BM in high concentrations, and is able to accumulate there [2]. With its wide spectrum of action, 5-HT, when introduced into the body, may affect proliferation both of HSC and of factors of the hematopoietic microenvironment.

Thus the results are strong evidence that 5-HT can regulate hematopoietic processes in their very earliest stages.

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