SEROTONIN OF HEMATOPOIETIC AND IMMUNOCOMPETENT TISSUES OF MAMMALS OF VARIOUS SPECIES

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High concentrations of serotonin, not attributable to the presence of blood in the tissues, were found by the ninhydrin fluorescence method in the bone marrow, lymph glands, and thymus of various species of mammals (dogs, cats, rabbits, guinea pigs, rats, and mice). Administration of exogenous serotonin increased its concentration in the tissues of these organs and also in the spleen. Reserpine sharply lowered the serotonin concentration in these tissues. It is postulated that endogenous serotonin of the hematopoietic and immunocompetent tissues participates in the local regulation of hematopoiesis, immunogenesis, and natural radioresistance. The corresponding effects of exogenous serotonin are probably connected with its direct penetration into the hematopoietic and immunocompetent tissues and to its action on tissue receptors.

KEY WORDS: serotonin; hematopoiesis; lymphoid tissues.

Serotonin has recently been found in the lymph glands and thymus of rats [5, 10]. The lymphoid tissues of other species of animals have not been investigated, nor has any attempt been made to study the bone marrow.

The object of this investigation was to determine the serotonin level in the above-mentioned mammalian tissues.

EXPERIMENTAL METHOD

Six species of mammals were studied (Table 1). The serotonin concentration was determined by the ninhydrin fluorescence method in the writers' modification [3, 4]. Proteins were precipitated with perchloric acid containing 7 mg/ml ascorbinate. Exclusion of ascorbic acid considerably reduced the concentration of serotonin determined, especially after high dilution of the samples with perchloric acid. This could be because of the decrease in the concentration of endogenous stabilizing substances under these conditions. The normal serotonin concentration in the blood of mice, according to the available data, varies from 0.2 μg/ml [8] to 3-5 μg/ml [11], evidently depending on the degree of stabilization of the serotonin at the stage of protein precipitation. The identity of the substance determined with serotonin was proved, not only by the high specificity of the method, but also by tests involving administration of reserpine. It will also be noted that the oxidation of endogenous serotonin with iodine (recommended by B. N. Manukhin) was just as complete as that of the crystalline amine (in the standard or on the addition of serotonin to tissue extracts). Exogenous serotonin, in the form of the creatinine-sulfate (Reanal, Hungary), was injected subcutaneously 15 min before sacrifice in a dose of 10 mg/kg calculated as the free base; reserpine was injected intraperitoneally in a dose of 5 mg/kg 18-24 h previously in a dose of 5 mg/kg (both substances in a volume of 10 ml/kg).

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TABLE 1. Serotonin Concentration in Mammalian Tissues (M ± m)

Animals	n	Weight	Sex	Blood (μg/ml)	μg/g: Spleen	Bone marrow	Lymph glands	Thymus
Dogs	6	9—30 kg	♂, ♀	0,269±0,060	6,66±1,80	4,80±0,47	4,03±1,34	0,897±0,166
Rabbits	5	1,8—3,6 kg	♂, ♀	6,82±0,28	42,37±13,48	1,64±0,23	1,79±1,23	3,61±1,94
Cats	5	0,5—0,9 kg	♀	6,58±1,68	16,82±2,06	1,83±0,89	0,96±0,13	1,58±0,39
Guinea pigs	2	2—3,3 kg	♀	1,20±0,02	5,08±0,36	1,19±0,90	2,95±0,54	2,59±1,15
Rats	4	0,4—0,6 kg	♂, ♀	1,14±0,55	3,75±0,26	1,80±1,10	1,75±0,73	1,26±0,43
Mice	4	155—205 g	♂, ♀	1,18±0,52	4,77±1,24	6,13±2,57	6,69±1,67	4,84±0,97
CBA	14	20—30 g	♀	3,85±0,64	10,92±1,18	12,88±2,01	5,02±0,44	5,41±0,96
C57Bl/6y	14	20—25 g	♀	4,70±0,37	15,32±1,62	15,50±2,55	7,03±1,88	4,81±0,99
C57Bl/6y	10	27—30 g	♀	3,79±2,26	14,15±5,13	26,2±4,04	7,63±0,88	9,77±1,28
Albino	10	17—25 g	♂, ♀	2,64±0,42	5,03±0,89	4,95±0,85	1,05±0,95	1,34±0,27

EXPERIMENTAL RESULTS

The results of the study of the serotonin concentration in the tissues are given in Table 1. Its concentration in the blood and spleen of the rabbits, guinea pigs, and rats was significantly higher than that given in the literature [11, 12] and than the level determined previously by the writers in the Ukraine [3, 4]. These differences were not found in the serotonin concentration in the tissues of dogs [8, 12] and noninbred albino mice [11]. Under the experimental conditions used, the serotonin concentration in human blood was also that usually found: 0.17 μg/ml.

The chief result of the investigations was the discovery of high concentrations of serotonin in the hematopoietic and immunocompetent tissues of the animals of all six species at different ages. Since the animals studied belong to five different families of two orders (rodents and carnivores), the results must evidently be regarded as characteristic for the class of mammals as a whole. The highest serotonin concentrations were found in inbred mice and rats.

The serotonin concentration in the tissues was not attributable to the presence of blood in them, for the concentration of the amine in the organs of dogs, guinea pigs, rats, and mice was higher than in the blood (by 3-18 times in dogs, by 4-5.7 times in rats). The values found were higher than in the brain and were approximately equal to, or even higher than (in rats) the concentration in the spleen and intestine, organs particularly rich in serotonin.

Serotonin biosynthesis actually in the hematopoietic and immunocompetent tissues seems unlikely, for tryptophan hydroxylase is found in only a few mammalian tissues (brain, intestine, liver) [7, 11]. There is direct evidence of the absence of a second enzyme of serotonin biosynthesis, a decarboxylase, in the bone marrow [12]. It can accordingly be postulated that the bone marrow, lymph glands, and thymus assimilate and accumulate serotonin from the blood stream. To test this hypothesis experiments were carried out in which exogenous serotonin was injected. The results are shown in Fig. 1: all the tissues investigated clearly assimilated serotonin. For the spleen this is in agreement with data in the literature [9]. The relative increase in the concentration of the amine in the lymph glands and, in particular, in the bone marrow and spleen was higher than in the blood. This is evidence of its true accumulation.

It is well known that the serotonin of many organs is exhausted by reserpine as a result of the blocking of active serotonin transport at the place where it is stored – the special vesicles. This characteristic effect of reserpine was observed now in all tissues. The decrease in the serotonin concentration after injection of reserpine was greatest in the spleen and blood, in agreement with data in the literature [3, 4, 11, 12]. Reserpine had an almost equally marked action on the serotonin concentration in the bone marrow, lymph glands, and thymus. The serotonin concentration of these organs fell to the same (bone marrow) or even a greater degree (lymph glands and thymus) than in the small intestine, lungs, and heart. The ability of the tissues both to accumulate large quantities of serotonin from the blood stream and to lose them under the influence of reserpine shows that the storage of serotonin in the bone marrow, lymph glands, and thymus

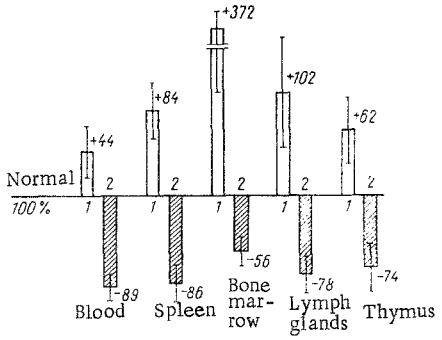

Fig. 1. Changes in serotonin concentration (in % of initial) in tissues of CBA mice under the influence of serotonin (1) and reserpine (2). All differences from normal statistically significant ($P < 0.05$).

takes place by a mechanism similar to or identical with that of storage in the spleen and platelets.

The presence of high concentrations of serotonin in the hematopoietic and immunocompetent tissues and their ability to assimilate and store the circulating amine are evidence of the important biological role of serotonin in these tissues. Exogenous serotonin is known to stimulate hematopoiesis [6, 13], to inhibit immunogenesis [2], and to give protection against radiation damage [1]. There are evidently grounds for suggesting that, first, endogenous serotonin may possess the same activity and, second, that these effects of exogenous serotonin are not only mediated by other systems [1], but are connected with its penetration directly into and its action on the hematopoietic and immunocompetent tissues. This last hypothesis is confirmed by the fact that serotonin accumulated in these tissues in doses giving a marked erythropoietic [13] and radioprotective [1] action and inhibiting antibody formation [2]. The penetration of serotonin into the tissues from the blood stream must lead not only to its storage, but also to its interaction with receptors. The last process arises also after mobilization of the stored serotonin of the bone marrow, spleen, lymph glands, and thymus under the influence of various factors. This hypothesis requires further experimental verification.

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