THE ACTION OF SEROTONIN ON THE MITOTIC ACTIVITY OF CERTAIN RAT ORGANS, AND BLOCKADE OF MONOAMINOXIDASE

(UDC 611.36-018.15:612.6.03]-063:615.787)

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(Presented by Active Member AMN SSSR N. A. Kraevskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 11, pp. 107-110, November, 1964
Original article submitted October 10, 1963

The power of serotonin to suppress cell division has been demonstrated in experiments on regeneration of the tadpole tail [5], and also in studies of HeLa cell cultures [7]. At the same time it has been established [17] that in young rats with a regenerating liver injections of serotonin increases the uptake of tritium-labelled thymidine by the parenchyma cells of the liver, i.e., it was shown that serotonin stimulates mitotic activity in the regenerating liver. The stimulating action of serotonin on the development of shoots of the oat has also been demonstrated [18]. Contradictory results have been obtained on the action of serotonin in inhibiting, on the one hand, the growth of various grafted rat and mouse tumors [3, 4, 23], and stimulating, on the other hand, myeloid hemopoiesis [6, 15, 25].

There are also indications of a possible involvement of serotonin in cell function in vivo. It is sometimes held [16] that serotonin should be considered an important growth factor whose action is to be attributed to its antioxidizing properties, and that it is also related to the activity of the somatotropic pituitary hormone.

On the other hand it has been proposed that the stimulation of hemopoiesis to which we have referred is not due to the direct action of serotonin on the bone marrow, but takes place through the activation of the adrenal cortex and through an enhanced secretion of cortico steroids [25]. Descriptions have been given of the hypertrophy and hyperfunction of the adrenals under the influence of serotonin [10, 22]. However according to the results obtained by other authors neither adrenalectomy or hypophysectomy causes appreciable changes in the distribution of serotonin among the various tissues [20, 21], and many of its pharmacological effects on adrenalectomized rats may even be enhanced [14].

At the same time from established data on the metabolism of serotonin in animals it may be supposed that its variable action on cell division depends upon differences in its rate of metabolism in the different tissues, i.e., in other words its action depends upon the variation in the activity of the enzyme monoaminoxidase, which is involved in the oxiditive deamination of 5-oxytryptamine and in the formation first of 5-oxyindoleacetaldehyde, and then of the terminal metabolite of serotonin-5-oxyindoleacetic acid. This idea is in line with the results of our experiments using blockade of monoaminoxidase in vivo before administration of serotonin to rats or mice tumors [4]; in this connection it has been shown that when monoaminoxidase has been blockaded the growth of certain tumors is more inhibited than it is under conditions when the activity of this enzyme is normal. We may therefore suppose that inhibition of cell division in a tumor is brought about by serotonin itself, and not by products of oxidation of monoaminoxidase which it has catalyzed.

The object of the present investigation has been to determine whether there is a relationship between the nature of the action of serotonin on cellular proliferation and the rate of its metabolism under the influence of monoaminoxidase.

METHOD

The experiments were carried out on male rats of an impure strain weighing about 200 g. Chlorhydrate of 5-oxytryptamine obtained from the laboratory of the chemistry of natural substances, Institute of Experimental and

Mitotic Activity in the Regenerating Liver, in the Duodenal Crypts, and in the Rat Corneal Epithelium after Intraperitoneal Injection of 30 mg/kg Serotonin

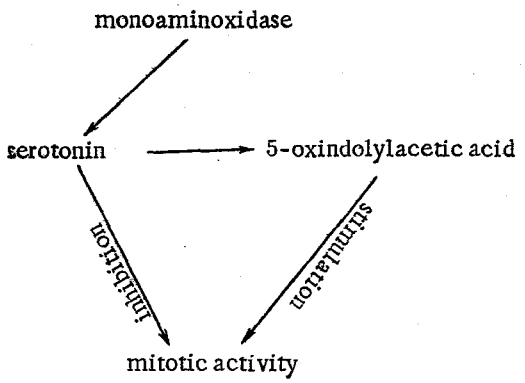
No. of experiment	Organ	Time of fixation	Number of rats		Mitotic index (percentage)		P
			Control	Experiment	Control	Experiment	
Action of serotonin under conditions of normal activity of monoaminoxidase							
1	Regenerating liver	In the morning	5	5	2.3	5.1	<0.001
2	Duodenum	" " "	5	5	7.8	8.5	0.45
3	Cornea	" " "	6	8	1.4	1.2	—
4	"	" " "	6	4	2.0	1.6	0.57
5	"	In morning (subcutaneous injection)	6	5	2.0	0.7	0.037
6	"	Evening	7	8	0.8	0.45	0.1
Action of serotonin under conditions of blockage of monoaminoxidase							
7	Cornea	Evening	7	5	0.8	0.07	<0.001
8	Duodenum	Morning	5	4	6.5	6.5	—
Control with iproniazid (without injection of serotonin)							
9	Cornea	Evening	7	4	0.8	0.24	0.005
10	Duodenum	Morning	5	4	6.5	6.2	—

Clinical Oncology, was mixed with physiological saline, and a single dose of 30 mg/kg was given intraperitoneally or subcutaneously. The material was fixed in formalin or with Carnoy's fluid, and subsequently stained with Feulgen or hematoxylin-eosin. We studied the mitotic activity of the regenerating liver after removal of two-thirds of the crypts, the duodenum, and the corneal epithelium. As a monoaminoxidase inhibitor we used iproniazid. The rats were decapitated and the organs fixed 2-3 h after the injection of serotonin either at 8 a.m. or 5 p.m. In experiments with regenerating liver serotonin was injected at 6 a.m., 70 h after resection. In the experiments with blockade of monoaminoxidase 100 mg/kg iproniazid was injected 20 h before the injection. Control material was fixed simultaneously with the material from each experimental group. For determination of the mitotic index we counted from 4,000 to 5,000 cells. The results were treated statistically by the method of Student.

RESULTS

Monoaminoxidase was shown to be present in most organs and tissues, where it localized in the mitochondria [2, 12]. It is well known that the liver shows a characteristic high monoaminoxidase activity [8, 26]. It is also known that serotonin exerts no toxic action on hepatic function even after mice have been injected with 100 mg/kg of 5-oxytryptamine chlorhydrate [1], but it does cause a great reduction in the amount of glycogen in the liver, and increases phosphorylase activity [11, 19]. Some authors [24] have observed a tendency for there to be a reduction in the activity of monoaminoxidase in actively proliferating tissues, particularly in regenerating rat liver. However, they have investigated the activity of the enzyme only 20 h after resection of the liver, i.e., principally before the onset of maximum proliferation; therefore quite possibly the activity of monoaminoxidase might have recovered by this time to the normal level.

Theoretically it would be expected that serotonin injected into a rat would be unlikely to inhibit mitosis in the liver, because it would become rapidly deaminated by monoaminoxidase. This expectation was fulfilled; serotonin caused no reduction of mitotic activity in the regenerating liver (see table), but, on the other hand, it markedly increased this activity. This result takes us beyond our initial supposition, but confirms the results which we have already mentioned as obtained by other authors concerning the stimulating action of serotonin on proliferation in the regenerating liver [17]; it suggests that in the liver not only is serotonin inactivated, but some other



The diagram illustrating proposed scheme of control by monoaminoxidase of the action of serotonin on cellular proliferation.

metabolite having mitotic stimulating properties is formed. It is quite probable that 5-oxyindoleacetic acid may serve as such a stimulator; large amounts of it may form rapidly from serotonin under the influence of monoaminoxidase (and of oxyindolylacetaldehyde-dehydrogenase of the liver); this substance is extremely closely related structurally to the heteroauxin plants – indolylacetic acid. The latter substance also possesses the power of stimulating mitotic activity in the regenerating liver [13]. There is therefore a relatively simple explanation of the contradictory results obtained in a study of the action of serotonin on various models of cellular proliferation, and it may be represented diagrammatically (see figure).

It would be expected that the reaction for serotonin on the part of the cells of the duodenal crypts should be of a special nature because the epithelium of the duodenum itself is particularly rich in enterochromaffin cells (cells of Kul'chitskii), which, in the modern view, are regions for the synthesis of serotonin. Therefore all the cells of the intestinal epithelium are without doubt adapted to the relatively high concentration of serotonin, which here functions as one of the intestinal hormones. In our experiment (see table) the duodenum was taken from rats with a regenerating liver; apparently it was on this account that the mitotic activity of the duodenal crypts was particularly high in animals of the control group which received no serotonin. In the experimental group the reaction of the cells to serotonin was weakly shown (the difference in the indices between the control and the experimental groups was not significant). The experiments on the action of serotonin on physiological regeneration of the corneal epithelium were repeated 4 times (see table, experiments Nos. 3-6); the material was fixed at various times of the day, either at 8 a.m. or 5 p.m., and the serotonin was injected subcutaneously instead of intraperitoneally (see table, experiment No. 5). In none of these experiments was any increase in mitotic activity observed in the cornea of rats receiving serotonin. On the other hand, in every case there was a reduction in the mitotic index, which reduction was however shown only in the experiment in which the serotonin was injected subcutaneously. Possibly the effect was due to a slower rate of absorption of the serotonin into the blood stream when it was injected in this way, so that there was then a reduced oxidation of this substance in the organ. Also, it has already been shown that there is some difference not only in the rate but also in the nature of the metabolism of serotonin, which depends upon the different ways it may be administered [9].

In experiments on blockade of monoaminoxidase in rat tissues (the blockade being produced by 100 mg/kg of iproniazid) and subsequent injection of serotonin, the mitotic index was studied only in the duodenal crypts and in the corneal epithelium. The reaction of the cells of these tissues to serotonin under conditions which favored reduction or complete suppression monoaminoxidase activity was again extremely varied (see table, experiments Nos. 7 and 8). Complete confirmation was obtained for the notion that in the experiments on the cornea the inhibitory effect of serotonin was related to the monoaminoxidase activity, because under these conditions serotonin caused almost complete cessation of cell division, and only a few late telophases remained. It was interesting that the cornea of the control group of rats (experiment No. 9), which received only iproniazid without a subsequent serotonin injection reacted by a smaller but nevertheless significant reduction of mitotic activity. It is highly probable that in this case the inhibition of the mitoses after the injection of iproniazid was caused by the accumulation in the cornea of serotonin from endogenous sources which had not undergone natural oxidation on account of the blockade of the monoaminoxidase. The cornea in experiment No. 6 was fixed at the same time.

In the crypts of the duodenum no reaction on the part of the proliferating cells occured in response to the injection of serotonin after iproniazid: the mitotic index of the experimental group coincided precisely with the control value. There have been reports of subsidiary routes for the biological inactivation and removal from the body of excess of 5-oxytryptamine, routes which involve the conjugation of this amine with sulfonic, sulfuric, or glucuronic acids and the formation of the corresponding esters where the most intense formation of esters is observed after oral administration of serotonin [9]. Possibly therefore cells of the epithelium of the digestive tract use this means of inactivation of serotonin without formation of 5-oxyindolylacetic acid extensively.

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