Metabolic Effect of Serotonin in the Rat.* (19821)

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In the course of a study of some of the pharmacological properties of serotonin (5-hydroxytryptamine), we were led to observe the effect of this substance on the oxygen consumption of the rat. Marked depression of oxygen consumption lasting for periods of more than an hour was observed. This effect is interesting since it is opposite to what would be predicted for a compound structurally related to the sympathomimetic amines.

Materials and Methods. Mature albino rats of the Wistar strain weighing 160 to 220 g and of both sexes were employed. The apparatus was the closed circuit type, in which calcium chloride and soda lime were used to absorb water and carbon dioxide, respectively. Measurement of oxygen consumption was made at ten minute intervals, adding oxygen from a reservoir by displacement with saturated sodium chloride solution. The solution was run in from a burette until the equality of pressure inside and outside the system was reestablished. The serotonin employed was the synthetic creatinine sulfate monohydrate complex, but all results are presented in terms of the free base. Injections of the aqueous solution were made intraperitoneally in a volume of 0.5 cc or less.

Results and Discussion. The observations are summarized in the table which gives the weight, sex, and preinjection oxygen consumption (in grams per hour per 100 g of rat, as the mean of 3 to 6 ten minute periods) for each rat, as well as the temperature and dose level of each experiment. The effect on the oxygen consumption is presented in terms of the maximum effect observed for any ten minute period and the time in minutes for the oxygen consumption to return from the initially observed effect to 50% of the maximal one. In these studies an effect of less than 20% or lasting less than 20 minutes is not considered significant. It can be seen that the minimally active dose is 0.5 to 1.0 mg/kg, since 1 of 3 rats at 0.5 mg/kg while 3 of 4 rats at 1.0 mg/kg gave positive responses. With higher doses, the effect is more pronounced, especially with regard to duration; a maximum intensity of depression of oxygen consumption is reached at 2 to 4 mg/kg. A representative experiment, that of rat No. 8 in the table, is shown in the figure.

TABLE I. Depression of Oxygen Consumption by Intraperitoneal Injection of Serotonin in the Albino Rat.

Rat No.	Wt, g	Sex	Temp., °C	O_2 consump./ hr/100 g rat, g	Dose, mg/kg	Max. change, %	Duration to 50% recovery, min.
1	200	F	24	.22	.5	—25	54
2	165	F	23	.21	.5	none	
3	200	M	28	.16	.5	—16	14
4	165	F	23	.24	1	—45	40
5	220	M	28	.19	1	—27	60
6	175	F	26	.14	1	—24	12*
7	210	F	28	.19	1	—29	30
8	205	F	24	.23	2	—53	55
9	160	F	25	.20	2	—30	22
10	200	M	23	.20	2	—48	49
11	215	M	23	.24	2	—48	57
12	165	F	24	.21	4	—40	50
13	185	F	28	.17	4	—44	70†
14	210	M	28	.23	4	—41	50
15	170	F	27	.23	4	—56	90
16	180	F	30	.16	4	+32	65
17	180	F	28	.23	4	—40	>120
18	195	F	28	.17	8	—60	65
19	220	F	29	.22	8	—45	>120‡
20	160	F	25	.23	8	—61	90

* 24 hr fasted. Preceded by rise to +39%.
† 48 hr fasted.
‡ Subcutaneous injection. Marked cyanosis.

These dose levels are far removed from those which show acute toxicity, the LD_{50} for the rat being about 50 mg/kg by intraperitoneal route(1), and 117 mg/kg subcutaneously(2), (see rat No. 19). The principal toxic symptom displayed was one of sporadic, spasmodic crawling movements during which the

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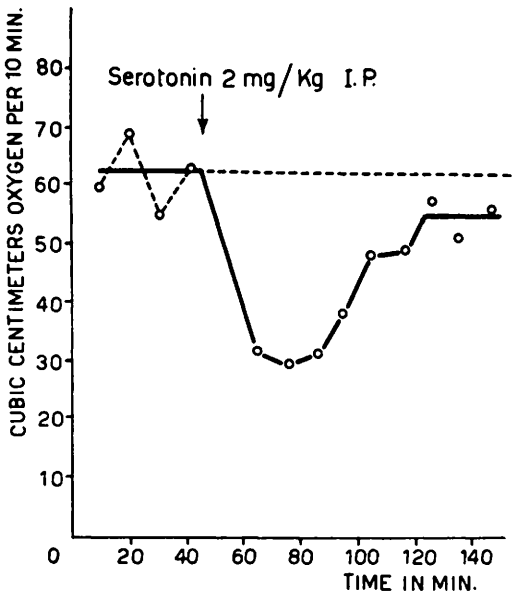

FIG. 1. Rat No. 8, female, 205 g.

animal appeared very disturbed. In general, respiratory frequency was somewhat depressed, changing from about 70 to 55 respirations per minute, but the decreased oxygen consumption has been observed also where the respiratory frequency was unchanged or even somewhat increased. About half of the rats displayed signs of irritation manifested by intense biting and scratching especially of the areas in the posterior regions. When this irritation was sufficiently pronounced, it could shorten the period of depressed oxygen consumption. This was the case with rat No. 9 in which the symptoms of irritation were greatest, and in which the period of metabolic depression was followed by one of increased oxygen consumption attaining a level of +38%.

The decrease in oxygen consumption is not necessarily related to the gross toxic reactions since two of the rats (Nos. 7 and 14) gave appreciable responses with very few toxic manifestations, while rat No. 16, the only animal which responded solely with an *increase* in the rate of oxygen consumption, showed the usual toxic symptoms. Rat No. 21, in which the temperature was measured before injection and during the period of maximal decrease in metabolic rate, showed a temperature drop from 37.4° to 33.2°.

The question naturally arises as to whether this effect represents a regulatory (hormonal) activity of serotonin or is rather a resultant of the pharmacological actions in the rat. The evidence in favor of the hormonal activity seems to rest with the wide distribution of the substance in both vertebrate and invertebrate species(3), and the fact that the activity is seen at a level of intraperitoneal injection as low as 1 mg/kg for a substance known to be rapidly inactivated by monoamine oxidase(2). The evidence in favor of the effect being pharmacological is somewhat stronger, in that no significant effect on oxygen consumption was seen in three other mammalian species: guinea pig, 20 mg/kg I.P.; rabbit, 10 mg/kg I.V., 4 and 50 mg kg I.P.; dog (under chloralose anesthesia), 2 mg/kg I.V., 10 mg/kg I.P. This species difference may be related to the low monoamine oxidase content of the rat kidney.

Although serotonin is known to have several peripheral pharmacological actions which might contribute to reduced oxygen consumption, it may be that the effect being observed here is in large part due to an action on the central nervous system or on the arterial vessels of the brain, and that the rat is peculiarly susceptible to this action. Rat No. 18 showed a temporary fore and hind leg paralysis as well as narcotic effects, the former lasting an hour, the latter two finally disappearing overnight. Two other observations implicating such a site of action are: 1) the rat shows a marked increase in acute toxicity to serotonin under anesthesia(4), and 2) the pial arteries of the rat have been shown to be sensitive to the serum vasoconstrictor(5).

A similar depression of the rate of oxygen consumption was observed with both tryptamine and histamine when injected intraperitoneally in the rat. The dosages required to obtain an effect with these two amines, both of which are closely related structurally to 5-hydroxytryptamine, were 10 times greater than with the latter, and the effect itself was different with respect both to the immediacy of onset and the duration. With tryptamine the lowered rate of oxygen consumption appeared only after a period of 20 to 25 minutes

following injection as compared with the immediate effect usually observed with serotonin. The period preceding an observable effect after histamine injection was even longer, and the duration of the effect was two hours or more. The toxic symptoms following administration of tryptamine were similar to those observed with serotonin; no toxic symptoms were seen following histamine injection.

Summary. Serotonin (5-hydroxytryptamine) has been found to depress the rate of oxygen consumption of the albino rat when injected intraperitoneally at dose levels as low as 1 mg/kg. A similar effect was observed with tryptamine and histamine (both of which are structurally related to serotonin) at much higher levels. This effect was not observed in the rabbit, guinea pig, or dog.

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1. Unpublished observations.

2. Freyburger W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. M., Swoap, O. F., and Vander Brook, M. J., *J. Pharm. and Exp. Therap.*, 1952, v105, 80.

3. Erspamer, V., and Asero, B., *Ricerca Scientifica*, 1951, v21, 2132.

4. D. I. Weisblat, private communication.

5. Brun, G. C., *Acta Pharm. et Toxic.*, 1950, v6, 74.

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Effect of Certain Carbohydrates on Nutritional Requirements of *Lactobacillus pentosus.* (19822)

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Lactobacillus pentosus, Strain 124-2, ferments both hexoses and pentoses(1-2). The organism has been grown on a synthetic medium containing glucose(3-5). However, when xylose replaced glucose in the synthetic medium, *L. pentosus* was reported not to grow(6). Growth was obtained when a supplement such as yeast extract was added and the existence of a "xylose factor" was postulated(6).

The present communication describes the study of an active principle in a liver extract supplement which promoted growth of *L. pentosus* on a synthetic medium containing either xylose or L-arabinose as a carbohydrate source.

Microbiological Methods. The organism used was *Lactobacillus pentosus,* Strain 124-2 (identical with *Lactobacillus plantarum* ATCC No. 8041). The synthetic medium employed was that described for the assay of nicotinic acid with *Lactobacillus arabinosus* (7) except that nicotinic acid was added and glucose was replaced by L-arabinose. Assays were carried out using either conventional microbiological technics in test tubes or by the pad plate technic(8) in which test solutions were added to filter paper discs and placed on agar plates seeded with *L. pentosus.* In the latter instance the diameter of the growth zone was used as a measure of the biological activity.

Results and Discussion. The data in Table I illustrate that *L. pentosus* failed to grow in a synthetic medium containing L-arabinose as a substrate unless a supplement such as liver extract or yeast was added. Small amounts of glucose duplicated the growth stimulatory effect of the liver extract. However, when the organism was cultured in the absence of L-arabinose, liver extract was inactive, and in experiment 1, for example, 1000 times as much glucose was now needed for growth.

Other sugars were tested for growth of *L. pentosus* on an L-arabinose medium. The data in Table II show that glucose and mannose were the most active while galactose and maltose had slight activity. Fructose and