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THE INFLUENCE OF pH ON SEROTONIN METABOLISM BY RAT TISSUE HOMOGENATES*

The current interest in the metabolism of hydroxyindoles has been brought about largely as a result of investigations of the pathways of serotonin (5-hydroxytryptamine) metabolism. Of the various enzyme systems involved in serotonin degradation, monoamine oxidase (MAO) has received the greatest attention, especially in view of its possible importance in controlling brain amine levels. According to present views MAO is the primary pathway of serotonin inactivation in most mammalian species.¹⁻⁴ This mode of detoxication, however, does not account for total serotonin breakdown by the intact animal. Under certain conditions, both in vitro and in vivo, other modes of serotonin inactivation have been reported; thus, the formation of the o-glucuronide and o-sulfate conjugates of serotonin has been demonstrated,⁵⁻⁷ and small amounts of such conjugates were found as urinary metabolites from rats.³ Weissbach et al⁷ have recently shown the importance of the glucuronide conjugating system in serotonin metabolism in the mouse, especially after blockade of MAO.

Some of the hydroxyindoleamines, such as bufotenin and psilocin, are rather poorly metabolized by MAO.⁸⁻⁹ Blaschko and Levine¹⁰ recently described the formation of colored oxidation products when various hydroxyindoles were incubated with preparations of gill plates from the *Mytilus edulis*. Horita and Weber¹¹ also observed a blue-colored product upon incubating psilocin with mammalian tissue homogenates, and from an examination of properties of the system suggested that cytochrome oxidase might be involved in this oxidation process.

In the present study the possibility of such a system inactivating serotonin was investigated. Since optimum oxidation of psilocin occurred at about pH 9.0, the influence of pH was also examined in this study. Rat tissue homogenates were found to be satisfactory as the source of the enzymes, and the effects of a MAO inhibitor and potassium cyanide were determined.

METHODS

Male Sprague-Dawley rats, 150-250 grams, were used exclusively as the source of the tissue homogenates. The liver, brain, kidney, and heart were ground with Teflon or glass homogenizers to a concentration of 20 per cent with ice cold distilled water. Incubations of liver homogenates were made for 30 minutes while the other preparations were shaken for 60 minutes. The following buffers were employed: Phosphate buffer (0.5M), pH 7.4; Tris buffer (0.5M), pH 8.1; Lysine buffer (0.5M), pH 8.8; and Glycine buffer (0.5M), pH 9.5. Each flask contained buffer, homogenate and 4 μ moles of serotonin creatinine sulfate. Potassium cyanide ($10^{-2}M$) and/or β -phenylisopropylhydrazine ($10^{-4}M$) were incorporated into some of the experimental procedures. In all cases the final volume of the incubation mixture was 3.0 ml. The degree of metabolism was determined by measuring the amount of serotonin remaining in the incubation flask after the designated time. Serotonin assays were performed according to the colorimetric method described by Udenfriend *et al.*¹²

The buffers used in these experiments were selected because of their abilities to hold the desired pH levels. They did not interfere with nor enhance the metabolism of serotonin as was evidenced by comparison

with other buffer systems. The possibility of spontaneous degradation of serotonin, especially at the higher pH levels, was carefully examined, but with the incubation times employed in these experiments, serotonin disappearance in buffer alone did not occur.

RESULTS

Liver. This tissue exhibited the greatest serotonin-metabolizing activity regardless of the pH of the medium. It should be noted that all incubations of liver homogenates were made for 30 minutes, while with the other preparations a 60 minute period was used. Under the conditions of these experiments liver homogenates metabolized about 80 per cent of the substrate present at pH 7.4. This was increased when pH was raised to 8.1 and 8.8, but at 9.5 metabolism decreased below 70 per cent.

In the presence of β -phenylisopropylhydrazine (PIH), $10^{-4}M$, the serotonin-metabolizing capacity of liver homogenate was reduced to zero at physiological pH. Slight amounts of metabolism occurred at the higher pH values but rarely exceeded 20 per cent. KCN, $10^{-2}M$, increased serotonin degradation, especially at the pH of 7.4 and 9.5. This was a consistent finding and was especially evident at all pH's when shorter incubation times were used so that less than 70-80 per cent of the serotonin was metabolized in the control (no KCN) preparations.

When both PIH and KCN were incorporated into the incubation mixture, the degree of serotonin disappearance was minimal, inhibiting an average of 11 per cent at pH 8.8. The influence of both pH and the inhibitors on serotonin metabolism by liver homogenates is shown in fig. 1, and the data and their standard deviations are listed in table 1.

Brain. Rat brain tissue homogenates proved to be considerably lower in serotonin metabolizing activity when compared to liver. The shape of the pH curve, however, did not differ too markedly from that of the liver, even in the presence of PIH or KCN. Complete inhibition was observed at all pH levels when both inhibitors were used simultaneously. The results of the brain homogenate studies are found in table 1.

Heart. Heart muscle proved to be quite different from either liver or brain in metabolizing serotonin. In the control homogenate the greatest activity was seen at pH 9.5, whereas with the other two tissues activity fell at this pH. An even greater difference was seen in the activity of the heart muscle preparation in the presence of PIH. While inhibition was complete at pH 7.4, at higher pH values a corresponding rise in serotonin breakdown was observed, and at pH 9.5 there was essentially no difference in activity between control and PIH-treated homogenates. KCN did not influence the activity of this preparation except at the highest pH where the metabolism of the substrate was reduced some 25 per cent. The combination of PIH and KCN produced complete block of metabolism. These results are shown in fig. 2 and in table 1.

Kidney. Only some 40 per cent of the serotonin was metabolized at 7.4 by control preparations of kidney homogenates. Like the heart, however, activity proceeded to rise with increased pH, and at 9.5 was capable of degrading 70 per cent of the substrate. In the presence of PIH the slope of the pH curve was similar to that seen with the heart. At pH 9.5, PIH exerted no influence on the metabolism of serotonin by kidney homogenates. Also similar to the heart, KCN showed greater inhibiting properties at the higher pH's, and at both 8.8 and 9.5,

considerable differences in activity between control and the KCN-treated preparation were evident. The preparations containing PIH and KCN, as with the other tissues, exhibited a minimal metabolism of serotonin (see table 1).

DISCUSSION

The results of this study indicate the presence of at least two enzyme systems in tissues of the rat which are capable of metabolizing serotonin. In the liver and brain, oxidative deamination by MAO is the primary mode of serotonin inactivation, even under unphysiologically high pH conditions. This is assumed to be the case because of the almost total inhibition of this reaction upon treatment with PIH, a specific MAO inhibitor. Although not described in the results, another MAO inhibitor, 2-phenylcyclopropylamine (SKF-385), was also found to inhibit this process by liver and brain homogenates at all pH levels. The heart and kidney, however, give evidence of two enzyme systems being involved in degrading serotonin. Predominant at pH 7.4 and 8.1 is MAO, as evidenced by marked inhibition by both PIH and SKF-385. At these pH levels control preparations of the heart tissue exhibited about the same activity as brain homogenates. Only after inhibition by the MAO inhibitors was it evident that some other system was also promoting the disappearance of serotonin. Even at pH 8.1 both kidney and heart homogenates showed PIH-resistant activity, and at 8.8 activity was about two-thirds of the non-inhibited control values. At pH 9.5 essentially no block was exerted by the MAO inhibitor. These results suggested that the same system which oxidized psilocin¹¹ was also capable of oxidizing serotonin. Preliminary work toward identifying this enzyme system indicates it to be cytochrome oxidase, especially because of its

KCN sensitivity, its activation by cytochrome c, and by the fact that purified pig heart cytochrome oxidase in the presence of cytochrome c is highly effective in oxidizing both psilocin and serotonin (unpublished results).

The values of percent metabolism of serotonin by heart and kidney homogenates in the presence of PIH or KCN, especially at the higher pH's, were at times variable as can be seen from their standard deviations. This variation was caused mainly by a small number of animals whose tissue preparations were inhibited to a greater extent by PIH than the majority of samples. Preparations of this type also responded poorly to KCN, usually resulting in considerably greater metabolism of serotonin than most KCN treated homogenates. The reason for these samples to act in this fashion is unclear. It appears that in these instances, MAO is the predominant system acting upon serotonin since PIH was quite effective in decreasing its metabolism. In the majority of cases, however, the reaction was resistant to PIH, but somewhat sensitive to KCN. Since the cytochrome oxidase system is dependent upon cytochrome c, it is possible that in these few instances where PIH was effective even at the higher pH's, cytochrome c may have been deficient and permitted the MAO to dominate the metabolism of the substrate. Evidence to this effect is seen in some of our current work; when cytochrome c is added to such a system the MAO inhibitors no longer block serotonin metabolism whereas KCN becomes quite effective.

Whatever the explanation, it is clear from these experiments that at the pH's of 8.8 and 9.5, rat heart and kidney homogenates have two systems operating to metabolize serotonin. That both MAO and the KCN-sensitive oxidase systems are active is seen by the degree of substrate breakdown

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In the presence of either PIH or KCN alone and its total inhibition in the presence of both inhibitors. It appears, however, that both systems may not be active simultaneously, especially at pH 9.5. This is indicated by the fact that at this pH the PIH treated preparation is capable of oxidizing the same amount of serotonin as does the control preparation.

If both systems were active the degree of serotonin metabolism by control homogenates should be greater than the PIH containing system. It also seems that at pH 9.5 the heart and kidney homogenates metabolize serotonin mainly via the KCN-sensitive oxidase system, provided that adequate cofactors be present. In the absence of such cofactors, or in the presence of KCN, the other enzyme system, MAO, becomes the primary pathway of serotonin inactivation. The reason why both systems are not simultaneously active to their full capacities cannot be explained by the present data.

Whether the KCN-sensitive system is an important pathway of serotonin inactivation in the intact animal is questionable, especially in view of its extremely high pH optimum. At best it may serve as a minor pathway of degradation under physiological conditions. Melisacs and Page³ indicate evidence of such a possible oxidation product in the urines of animals treated with C-¹⁴ serotonin. Perhaps a more practical aspect of the present findings is the possibility of the KCN-sensitive enzyme interfering with investigations of 5-hydroxytryptophan (5HTP) decarboxylase activity when kidney or heart homogenates are employed as the source of enzyme. In some instances 5HTP decarboxylase activity has been measured by incubating the homogenate with 5HTP at pH 8.0 - 8.1 in the presence of a MAO inhibitor and under aerobic conditions. We find that this procedure

does not give a true representation of the amount of serotonin synthesized since the KCN-sensitive enzyme can degrade a part of the formed product. In the presence of a MAO inhibitor and KCN ($10^{-3}M$), or under totally anaerobic conditions, the amount of serotonin recovery is increased considerably. These precautions are therefore necessary in using such homogenates as the source of 5HTP decarboxylase.

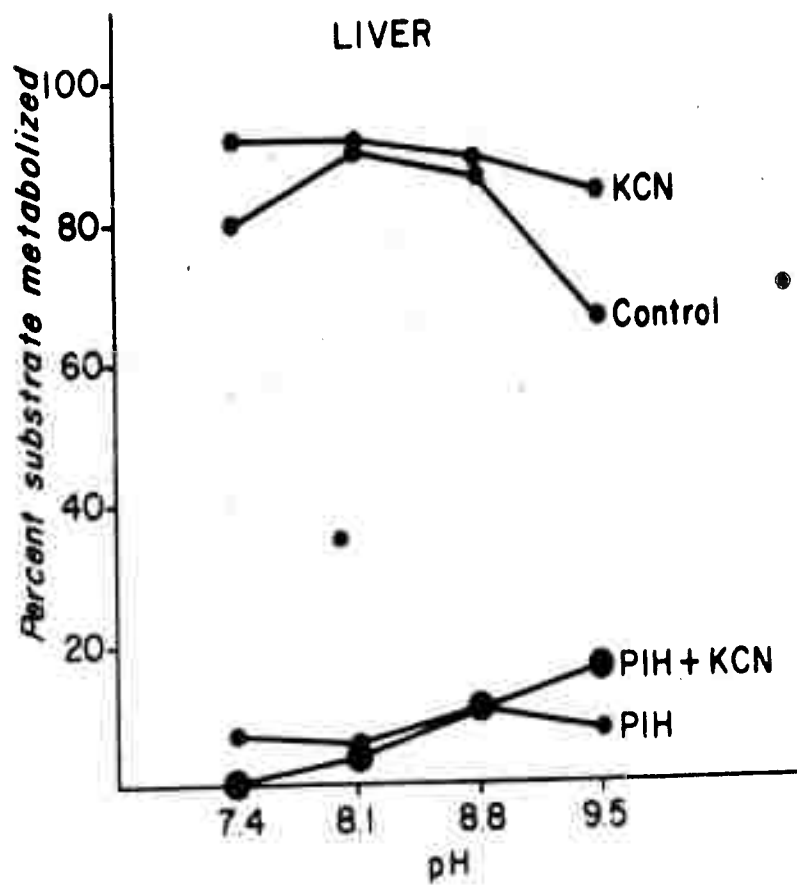
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TABLE 1. THE INFLUENCE OF pH AND INHIBITOR SUBSTANCES ON SEROTONIN METABOLISM

Values are expressed as mean percent metabolism $\pm$ standard deviation of the mean. Figures in parentheses indicate number of determinations in each group of experiments.

	pH	Liver	Brain	Heart	Kidney
Control	7.4	78 $\pm$ 3 (6)	60 $\pm$ 4 (6)	61 $\pm$ 9 (7)	41 $\pm$ 5 (7)
	8.1	89 $\pm$ 3 (8)	69 $\pm$ 3 (6)	73 $\pm$ 9 (7)	42 $\pm$ 3 (8)
	8.8	86 $\pm$ 6 (5)	64 $\pm$ 7 (5)	71 $\pm$ 10 (9)	48 $\pm$ 6 (9)
	9.5	66 $\pm$ 2 (6)	65 $\pm$ 4 (6)	83 $\pm$ 4 (8)	69 $\pm$ 4 (9)
PIH	7.4	0 (5)	0 (6)	0 (4)	0 (4)
	8.1	4 $\pm$ 4 (13)	0 (4)	20 $\pm$ 7 (7)	13 $\pm$ 4 (9)
	8.8	11 $\pm$ 10 (11)	12 $\pm$ 7 (5)	56 $\pm$ 23 (11)	33 $\pm$ 10 (14)
	9.5	17 $\pm$ 9 (8)	21 $\pm$ 10 (6)	81 $\pm$ 7 (6)	66 $\pm$ 4 (6)
KCN	7.4	92 $\pm$ 2 (5)	65 $\pm$ 9 (5)	64 $\pm$ 15 (7)	49 $\pm$ 9 (6)
	8.1	91 $\pm$ 3 (6)	74 $\pm$ 3 (7)	73 $\pm$ 20 (7)	45 $\pm$ 2 (6)
	8.8	90 $\pm$ 4 (6)	67 $\pm$ 6 (6)	68 $\pm$ 16 (11)	31 $\pm$ 6 (10)
	9.5	85 $\pm$ 13 (9)	54 $\pm$ 15 (6)	58 $\pm$ 27 (9)	27 $\pm$ 6 (6)
PIH + KCN	7.4	7 $\pm$ 3 (4)	0 (4)	0 (4)	5 $\pm$ 2 (4)
	8.1	6 $\pm$ 3 (5)	0 (4)	3 $\pm$ 4 (5)	6 $\pm$ 6 (5)
	8.8	11 $\pm$ 5 (5)	0 (4)	7 $\pm$ 4 (7)	12 $\pm$ 6 (6)
	9.5	8 $\pm$ 7 (5)	0 (4)	5 $\pm$ 5 (6)	8 $\pm$ 7 (6)



Figure

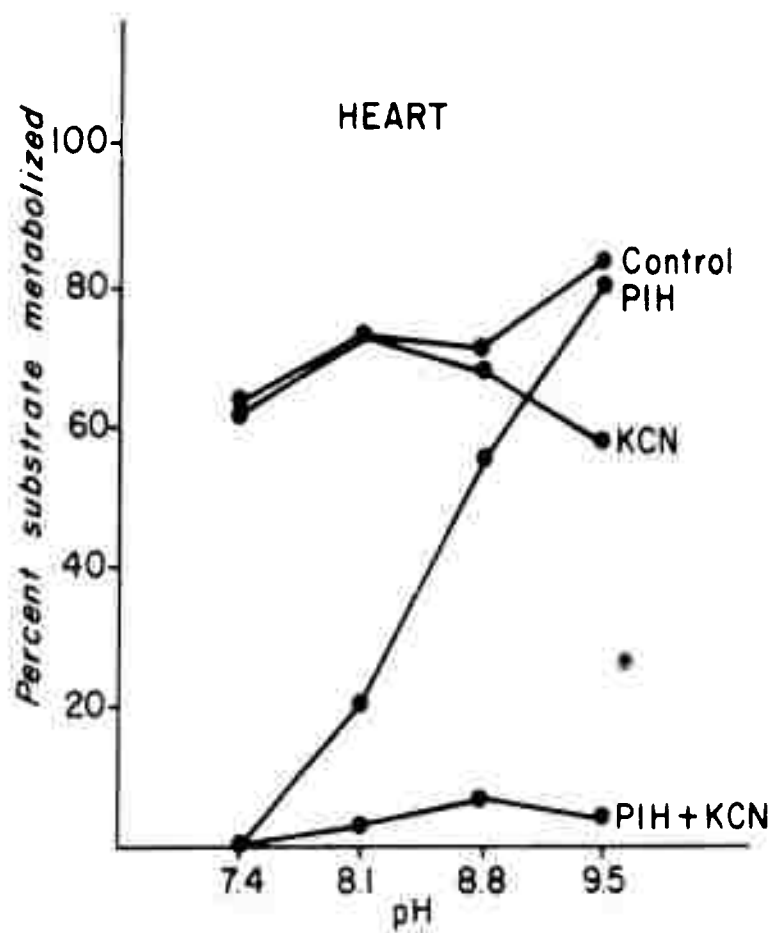


Figure 2

Footnotes

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Legends to Figures

Figure 1. The metabolism of serotonin by rat liver homogenates at various pH levels and in the presence of (1) no drug, (2) PIH ($10^{-4}M$), (3) KCN ($10^{-2}M$) and (4) PIH and KCN.

Figure 2. The metabolism of serotonin by rat heart homogenates at various pH levels and in the presence of (1) no drug, (2) PIH ($10^{-4}M$), (3) KCN ($10^{-2}M$) and (4) PIH and KCN.

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