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

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Abstract—The metabolism of serotonin by rat tissue homogenates at varying pH levels has been investigated. Liver and brain preparations showed greatest serotonin-metabolizing activity at slightly alkaline levels, pH 8·1, while heart and kidney homogenates exhibited highest activity at pH 9·5. In the presence of an inhibitor of monoamine oxidase, all preparations were effectively blocked at pH 7·4, but almost no protection against the disappearance of serotonin was present at pH 9·5 with the heart and kidney preparations. KCN, which did not affect serotonin metabolism at physiological levels of pH, inhibited considerably the activity of kidney and heart preparations. It is concluded that under the conditions of these experiments two enzyme systems are involved in the metabolism of serotonin. One, which is most active in liver and brain, is monoamine oxidase. The other, most active in heart and kidney, is a cyanide-sensitive enzyme which probably acts on the hydroxyl group of serotonin.

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; 5HT) metabolism. Of the various enzyme systems involved in the degradation of 5HT, 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 enzyme concerned with the inactivation of 5HT in most mammalian species.[1–4] This mode of detoxication, however, does not account for all degradation of 5HT by the intact animal. Under certain conditions, both *in vitro* and *in vivo*, other modes of inactivation of 5HT 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 al.*[7] have demonstrated the importance of the glucuronide-conjugating system in the metabolism of 5HT by the mouse, especially after the blockade of MAO.

Some of the hydroxyindoleamines, such as bufotenin and psilocin, are rather poorly metabolized by MAO.[8–9] Blaschko and Levine[10] have described the formation of colored oxidation products when various hydroxyindoles were incubated with preparations of gill plates from *Mytilus edulis*. Also, Horita and Weber[11] 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.

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In the present study the possibility of such a system inactivating serotonin has been 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 an inhibitor of MAO and of potassium cyanide were determined.

METHODS

Male Sprague–Dawley rats, 150–250 g, 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 min while the other preparations were shaken for 60 min. The following buffers were employed: phosphate buffer (0·5 M), pH 7·4; Tris buffer (0·5 M), pH 8·1; lysine buffer (0·5 M), pH 8·8; and glycine buffer (0·5 M), pH 9·5. Each flask contained buffer, homogenate and 4 μmoles of serotonin–creatinine sulfate. Either potassium cyanide (10^{-2} M) or β-phenyl*iso* propylhydrazine (10^{-4} M), or both, were used in 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 5HT remaining in the incubation flask after the designated time. Assays for 5HT 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 neither interfered with nor enhanced the metabolism of 5HT, as was evidenced by comparison with other buffer systems. The possibility of spontaneous degradation of 5HT, especially at the higher pH levels, was carefully examined, but with periods of incubation employed in these experiments, disappearance of 5HT did not occur in buffer alone.

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 min, while with the other preparations a 60-min 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 the pH levels were raised to 8·1 and 8·8, but at pH 9·5 the metabolism of 5HT was decreased.

In the presence of β-phenyl*iso*propylhydrazine (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 pH levels of 7·4 and 9·5. This was a consistent finding and was especially evident at pH levels when shorter incubation times were used; thus, less than 70 to 80 per cent of the 5HT was metabolized in the control (no KCN) preparations.

When both PIH and KCN were incorporated into the incubation mixture, the disappearance of 5HT was minimal (an average of 11 per cent at pH 8·8). The influence of both pH and the inhibitors on the metabolism of 5HT by liver homogenates are 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

With respect to the metabolism of 5HT, heart muscle proved to be quite different from either liver or brain. 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

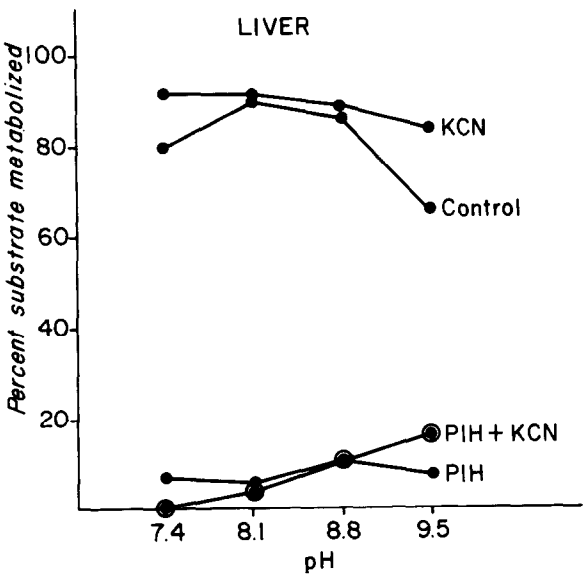

FIG. 1. The metabolism of serotonin by rat liver homogenates at various pH levels and in the presence of (1) no drug, (2) PIH (10^{-4}M), (3) KCN (10^{-2}M) and (4) PIH and KCN.

PIH. While inhibition was complete at pH 7·4, at higher pH values a corresponding increase in the degradation of 5HT was observed; thus, 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 levels, at which the metabolism of the substrate was reduced by about 25 per cent. The combination of PIH and KCN caused a complete inhibition of the metabolism of 5HT. These results are shown in Fig. 2 and in Table 1.

Kidney

Only some 40 per cent of the serotonin was metabolized at pH 7·4 by control preparations of kidney homogenates. As with the heart, however, the metabolic activity rose with increasing pH, and, at pH 9·5, this tissue homogenate degraded 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 5HT by kidney homogenates. As with the cardiac tissue homogenate, KCN exerted greater inhibiting properties at the higher levels of pH, and at both pH 8·8 and 9·5, respectively, 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).

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

(Values are expressed as mean per cent metabolism ± standard deviation of the mean. Figures in parentheses indicate the number of determinations in each group of experiments.)

	pH	Liver	Brain	Heart	Kidney
Control	7·4	78±3 (6)	60±4 (6)	61±9 (7)	41±5 (7)
	8·1	89±3 (8)	69±3 (6)	73±9 (7)	42±3 (8)
	8·8	86±6 (5)	64±7 (5)	71±10 (9)	48±6 (9)
	9·5	66±2 (6)	65±4 (6)	83±4 (8)	69±4 (9)
PIH	7·4	0 (5)	0 (6)	0 (4)	0 (4)
	8·1	4±4 (13)	0 (4)	20±7 (7)	13±4 (9)
	8·8	11±10 (11)	12±7 (5)	56±23 (11)	33±10 (14)
	9·5	17±9 (8)	21±10 (6)	81±7 (6)	66±4 (6)
KCN	7·4	92±2 (5)	65±9 (5)	64±15 (7)	49±9 (6)
	8·1	91±3 (6)	74±3 (7)	73±20 (7)	45±2 (6)
	8·8	90±4 (6)	67±6 (6)	68±16 (11)	31±6 (10)
	9·5	85±13 (9)	54±15 (6)	58±27 (9)	27±6 (6)
PIH + KCN	7·4	7±3 (4)	0 (4)	0 (4)	5±2 (4)
	8·1	6±3 (5)	0 (4)	3±4 (5)	6±6 (5)
	8·8	11±5 (5)	0 (4)	7±4 (7)	12±6 (6)
	9·5	8±7 (5)	0 (4)	5±5 (6)	8±7 (6)

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 inactivation of 5HT, even under unphysiologically high pH conditions. This finding is interpreted as signifying

the almost total inhibition of this reaction upon treatment with PIH, a specific MAO-inhibitor. Although not described in the results, another inhibitor of MAO, 2-phenylcyclopropylamine (SKF-385), also inhibited this process by liver and brain homogenates at all pH levels. In the heart and kidney, however, the evidence obtained suggested that two enzyme systems are involved in degrading serotonin. Predominant

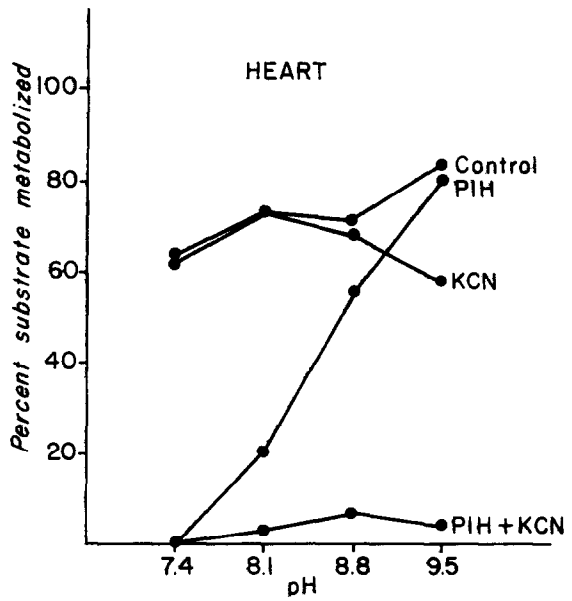

FIG. 2. The metabolism of serotonin by rat heart homogenates at various pH levels and in the presence of (1) no drug, (2) PIH (10^{-4}M), (3) KCN (10^{-2}M) and (4) PIH and KCN.

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 also was promoting the disappearance of serotonin. Even at pH 8·1, both kidney and heart homogenates exhibited PIH-resistant activity, and, at pH 8·8, the activity was about two-thirds of those 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 oxidizes psilocin[11] also is capable of oxidizing serotonin. Preliminary attempts to identify this enzyme system indicate that it may be cytochrome oxidase, especially because of its KCN-sensitivity and its activation by cytochrome *c*; such a conclusion is supported by the finding that purified pig heart cytochrome oxidase, in the presence of cytochrome *c*, is highly effective in oxidizing both psilocin and serotonin (unpublished results).

The extent of the metabolism of 5HT by heart and kidney homogenates in the presence of PIH or KCN, especially at the higher levels of pH, were at times variable, as can be seen from their standard deviations. This variation was caused mainly by a few animals, the tissue preparations of which were inhibited to a greater extent by PIH than were those of the majority of the animals. Preparations of this type also

responded poorly to KCN, a circumstance which usually resulted in a considerably greater metabolism of serotonin than with most KCN-treated homogenates. The explanation for these findings is not yet apparent. It appears that in these instances, MAO is the predominant system acting upon 5HT, since PIH was quite effective in decreasing its metabolism. In the majority of cases, however, the metabolism of 5HT 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 in which PIH was effective even at the higher levels of pH, 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 the metabolism of 5HT, whereas KCN becomes quite effective.

Whatever the explanation, it is clear from these experiments that at pH levels of 8·8 and 9·5, rat heart and kidney homogenates contain two systems which can metabolize serotonin. That both MAO and the KCN-sensitive oxidase systems are active is seen by the degree of substrate breakdown 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 are present. In the absence of such cofactors, or in the presence of KCN, the other enzyme system, MAO, becomes the primary pathway of 5HT-inactivation. The reasons why both systems are not simultaneously active to their full capacities cannot be explained by the present data. Similar observations were made by Friedenwald and Herrmann[13] in their studies on the oxidation of epinephrine by rabbit intestinal mucosa. In the absence of cytochrome *c* the oxidation of epinephrine could be completely inhibited by amphetamine, an inhibitor of MAO *in vitro*. When cytochrome *c* was included in the mixture, amphetamine was no longer effective, whereas cyanide completely suppressed the oxidation process. These authors concluded that the addition of cytochrome *c* activated the cytochrome system to oxidize epinephrine into an intermediate which acted as an inhibitor of MAO. This would provide an explanation for the inability of amphetamine to block the MAO in the presence of an active cytochrome system. At present, however, we have evidence that such is not the case in our studies reported here.

Whether the KCN-sensitive system is an important pathway of inactivation of 5HT 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. McIsaacs and Page[3] have presented evidence of such a possible oxidation product in the urines of animals treated with serotonin labelled with carbon-14. Perhaps a more practical aspect of the present findings is the possibility of the KCN-sensitive enzyme interfering with investigations of the activity of 5-hydroxytryptophan (5HTP) decarboxylase, 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 an inhibitor

of MAO and under aerobic conditions. We find that this procedure does not give a true representation of the amount of serotonin produced, since the KCN-sensitive enzyme can degrade a part of the product formed. In the presence of both an inhibitor of MAO and KCN (10^{-3} M), or under totally anaerobic conditions, the recovery of 5HT is considerably increased. Accordingly, these precautions are necessary when such homogenates are used as the source of 5HTP decarboxylase.

REFERENCES

1. S. UDENFRIEND, *Vitamins and Hormones* **17**, 133 (1959).
2. A. N. DAVISON, *Physiol. Revs.* **38**, 729 (1958).
3. W. M. MCISAAC and J. H. PAGE, *J. Biol. Chem.* **234**, 858 (1959).
4. D. KEGLERIC, Z, SUPEK, S. KREDEN, S. ISRIC, S. KECKES and A. KISK, *Biochem. J.* **73**, 53 (1960).
5. B. T. CHADWICK and J. H. WILKINSON, *Biochem. J.* **68**, 1p (1958).
6. G. CURZON, *Arch. Biochem. Biophys.* **66**, 497 (1957).
7. H. WEISSBACH, W. LOVENBERG, B. G. REDFIELD and S. UDENFRIEND, *J. Pharmacol.* **131**, 26 (1961).
8. V. ERSPAMER, R. FERRINI and A. GLASSER, *J. Pharmacol.* **12**, 761 (1960).
9. P. K. GESSNER, P. A. KHAIRALLAH, W. M. MCISAAC and I. H. PAGE, *J. Pharmacol.* **130**, 126 (1960).
10. H. BLASCHKO and W. G. LEVINE, *Brit. J. Pharmacol.* **15**, 625 (1960).
11. A. HORITA and L. J. WEBER, *Biochem. Pharmacol.* **7**, 47 (1961).
12. S. UDENFRIEND, H. WEISSBACH and B. B. BRODIE, *Meth. Biochem. Anal.* **6**, 95 (1958).
13. J. S. FRIEDENWALD and H. HERRMANN, *J. Biol. Chem.* **146**, 411 (1942).