5-Hydroxytryptamine metabolism in rat brain and liver homogenates

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1. The metabolism of 5-hydroxyindoleacetaldehyde derived from 5-hydroxytryptamine incubated with tissue homogenates was studied as an indicator of aldehyde dehydrogenase and alcohol dehydrogenase activities.

2. In liver and brain from rats, there were indications of the presence of one or more aldehyde dehydrogenases which were stimulated by NAD^+ to a greater extent than by $NADP^+$.

3. In liver from rats, there were indications of the presence of one or more alcohol dehydrogenases, which were stimulated by NADH to a greater extent than by NADPH.

4. In brain from rats, there were indications of the presence of one or more alcohol dehydrogenases which were stimulated by NADPH to a greater extent than by NADH.

Eccleston, Moir, Reading & Ritchie (1966) reported that 5-hydroxytryptamine (5-HT) was converted to 5-hydroxytryptophol by way of 5-hydroxyindoleacetaldehyde in rat and human brain homogenates. These investigators found that the conversion in brain was effected by an alcohol dehydrogenase which depended on NADPH (Eccleston, Moir, Reading & Ritchie, 1966) rather than NADH as in liver (Kveder, Iskric & Keglevic, 1962 ; Feldstein & Wong, 1965a, b). It was reported also that the conversion of 5-HT to 5-hydroxyindoleacetic acid (5-HIAA) by way of 5-hydroxyindoleacetaldehyde in rat brain homogenates was more effective in the presence of $NADP^+$ than NAD^+ (Eccleston, Moir, Reading & Ritchie, 1966), although another investigator (Deitrich, 1966) found that NAD^+ was the more effective. The effects of the coenzymes on 5-HT metabolism in both rat liver and brain homogenates were re-investigated and the results reported herein.

Methods

Male rats, Sprague-Dawley descendants, 150–165 g, were obtained from Charles River Laboratories. A 2% brain or 0.5% liver homogenate was prepared in 0.25 M-sucrose, with cooling. For each tissue sample, five incubation flasks were set up, control and one flask for each of the four coenzymes: NAD^+, $NADP^+$, NADH and NADPH. The final incubation mixture contained 10 μg of 5-hydroxytryptamine-^{14}C creatinine sulphate hydrate, 1% brain or 0.25% liver, 1 mg of coenzyme, 0.125 M-sucrose and 0.25 M-phosphate buffer, pH 7.4, in a total volume of

1 ml. and the incubation was carried out for 80 min at 37° C with shaking in air. The 5-HT-^{14}C salt (specific activity, 98 μc/mg) was obtained from Nuclear-Chicago, Inc. The coenzymes used, except where noted, were obtained from Sigma Chemical Co. The analysis of metabolites was by the previously described method (Feldstein & Wong, 1965a, b), which combines solvent extraction procedures with paper chromatographic separation of 5-hydroxytryptophol and 5-hydroxyindoleacetaldehyde.

Results

The incubation of a 0.25% liver homogenate (Table 1) with 5-HT for 80 min under the conditions described indicated that about 68% of the 5-HT was metabolized by monoamine oxidase (MAO) to 5-hydroxyindoleacetaldehyde with subsequent formation of 5-hydroxytryptophol and 5-HIAA. Approximately the same percentage of 5-HT—62%—was metabolized by 1% brain homogenate (Table 2). Liver MAO therefore was about 4 times more active than brain MAO. In a series of 10 min incubations, where product formation was linear with respect to time, liver MAO (six rats) was again 4 times more active than brain MAO (six rats).

In liver incubation mixtures, 5-HIAA formation occurred in the absence of exogenous coenzymes (Table 1), which indicated aldehyde dehydrogenase activity. If

TABLE 1. *Metabolism of 5-hydroxytryptamine in rat liver homogenates*

5-HT metabolites	Mean percentage (range observed in five rats) of 5-HT metabolized in 80 min				
	Control	NAD⁺	NADP⁺	NADH	NADPH
Total products formed	68 (60–78)	71 (61–84)	71 (57–83)	65 (55–81)	60 (42–79)
5-Hydroxyindole-acetaldehyde	33 (16–48)	1 (0–1)	12 (1–21)	6 (5–8)	18 (4–31)
5-Hydroxytryptophol	6 (4–10)	2 (0–4)	5 (3–10)	36 (22–47)	11 (4–29)
5-HIAA	24 (7–46)	67 (55–83)	50 (28–75)	15 (7–26)	27 (7–60)

The final incubation mixture contained (per ml.): 10 μg of 5-HT creatinine sulphate hydrate; 0·25% liver homogenate; 0·125 M-sucrose; 0·25 M-phosphate buffer at pH 7·4; 1 mg of coenzyme.

TABLE 2. *Metabolism of 5-hydroxytryptamine in rat brain homogenates*

5-HT metabolites	Mean percentage (range of five rats) of 5-HT metabolized in 80 min				
	Control	NAD⁺	NADP⁺	NADH	NADPH
Total products formed	62 (48–73)	59 (45–80)	54 (39–73)	54 (38–73)	55 (38–80)
5-Hydroxyindole-acetaldehyde	38 (23–49)	21 (11–29)	30 (22–42)	28 (15–38)	24 (16–27)
5-Hydroxytryptophol	3 (3–3)	2 (2–3)	3 (2–5)	4 (3–5)	13 (8–21)
5-HIAA	13 (12–15)	29 (21–39)	14 (10–18)	16 (13–23)	11 (8–16)

The final incubation mixture contained (per ml.): 10 μg of 5-hydroxytryptamine creatinine sulphate hydrate; 1% brain homogenate; 0·125 M-sucrose; 0·25 M-phosphate buffer at pH 7·4; 1 mg of coenzyme.

the homogenate was prepared at room temperature or preincubated at 37° C for 15 min, instead of being prepared with cooling and used directly, total product formation (hence MAO activity) was not altered but 5-HIAA formation was reduced ; exogenous NAD^+ reactivated the aldehyde dehydrogenase under these conditions. The addition of NAD^+ to the incubation mixture prepared from the cooled homogenates (Table 1) also greatly increased 5-HIAA formation ; $NADP^+$ caused a moderate increase. The source of the NAD^+ determined in part the rate of 5-HIAA formation (Table 3). 5-Hydroxytryptophol formation was low in the absence of exogenous coenzymes (control values, Table 1). NADH moderately increased 5-hydroxytryptophol formation (Table 1) ; whereas, NADPH caused only a slight increase ($P < 0.05$).

In rat brain incubation mixtures (Table 2) the formation of 5-HIAA was moderately increased by the addition of NAD^+. If an adjustment for the difference in total product formation is made, a small increase ($P < 0.05$) with $NADP^+$ in the relative amount of 5-HIAA formed is evident (Tables 2 and 4). Formation of 5-hydroxytryptophol was slightly but not significantly increased by NADH, and moderately increased ($P < 0.05$) by NADPH (Table 2). $NADP^+$ partially prevented the NADPH-induced increase in 5-hydroxytryptophol formation, and there was a simultaneous increase in 5-HIAA formation (Table 4).

Discussion

The formation of 5-HIAA from 5-hydroxyindoleacetaldehyde derived from 5-HT in guinea-pig (Weissbach, Redfield & Udenfriend, 1957), rat (Table 1; Deitrich, 1966) and human (Feldstein, Williamson & Tedeschi, unpublished) liver incubations

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TABLE 3. *Conversion of 5-hydroxytryptamine to 5-HIAA in rat liver homogenates as a function of NAD+ source*

Source of NAD+	Mean percentage (range) of 5-hydroxytryptamine converted to 5-hydroxy-indoleacetaldehyde and 5-HIAA	5-HIAA formed expressed as the mean percentage (range) of the total products formed
Control	7·9 (7·5–8·3)	8 (6–8)
Company A	6·9 (6·6–7·2)	29 (25–34)
Company B	7·2 (6·8–7·6)	47 (41–54)
Company C—Lot 1	6·9 (6·4–7·3)	57 (48–66)
Company C—Lot 2	7·1 (6·9–7·5)	68 (63–73)

The final incubation mixture contained (per ml.): 10 μg of 5-hydroxytryptamine creatinine sulphate hydrate; 0·25% of liver homogenate; 0·125 M-sucrose; 0·25 M-phosphate buffer at pH 7·4; 1 mg of NAD^+. Incubation time was 5 min and product formation was linear with respect to time.

TABLE 4. *5-Hydroxytryptamine metabolism in rat brain homogenates as a function of NADPH and varying concentrations of NADP+*

5-Hydroxytryptamine metabolites	mg of NADP+/ml. in the presence of 1 mg of NADPH/ml. incubation mixture	Relative percentage of products formed in 80 min		
		0	0·5	1·0	2·0
5-Hydroxyindole-acetaldehyde		52	50	53	55
5-Hydroxytryptophol		21	16	12	9
5-HIAA		27	34	35	36

was stimulated to a greater extent by NAD^+ than by $NADP^+$. These results suggest that there may be two aldehyde dehydrogenases in liver, a NAD^+-linked and a $NADP^+$-linked enzyme. There is a precedent for the separation of two enzymes which yield the same product, the NAD^+- and $NADP^+$-linked isocitrate dehydrogenase (Plaut & Aogaichi, 1967). Stimulation of 5-HIAA formation may be due, however, to a single enzyme which functions with both NAD^+ and $NADP^+$ or which functions with NAD^+ derived by rapid dephosphorylation of $NADP^+$ (Sanadi, 1952; Stollar & Kaplan, 1961; Jouany, 1965).

We have also found that NADH stimulated 5-hydroxytryptophol formation in rat (Feldstein & Wong, 1965a, b; Table 1) and human (Feldstein, Williamson & Tedeschi, unpublished) liver homogenates. NADPH was only slightly less effective than NADH with human liver (Feldstein, Williamson & Tedeschi, unpublished) but considerably less effective than NADH with rat liver (Table 1). Again, we may consider the possibility of two distinct enzymes in liver, a NADH-linked and a NADPH-linked alcohol dehydrogenase, although a single enzyme which functions with both coenzymes should also be considered (Dalziel & Dickinson, 1965).

In rat (Table 2) and human (Feldstein, Williamson & Tedeschi, unpublished) brain homogenates, we have found that 5-HIAA formation was moderately stimulated by NAD^+ and to a lesser extent by $NADP^+$. The quality of the NAD^+ may explain the difference between our results and those obtained by Eccleston *et al.* (1966), because enzymic activity was dependent on the source of the coenzyme (Table 3). The NAD^+ used in our studies was rated greater than 99% pure by the company and had maximal activity available. Also the presence of NAD^+ in commercial samples of $NADP^+$ may have played a part (Dalziel & Dickinson, 1965); the $NADP^+$ used in our experiments was more than 99% pure and the company noted that the presence of NAD^+ could not be detected. Our results agreed with those of Deitrich and co-workers (Deitrich, 1966; Erwin & Deitrich, 1966), who also found greater stimulation of 5-HIAA formation with NAD^+ than with $NADP^+$ in rat brain homogenates; their isolated enzyme, in fact, showed no activity with $NADP^+$. Hence, too, we may consider the possibility of two distinct enzymes in brain, a NAD^+-linked and a $NADP^+$-linked aldehyde dehydrogenase, although the possibility of a single enzyme which functions with both NAD^+ and $NADP^+$ cannot be excluded.

In agreement with the findings of Eccleston, Moir, Reading & Ritchie (1966) we have found that NADPH moderately stimulated 5-hydroxytryptophol formation in rat brain homogenates (Table 2). We have also found that $NADP^+$ added to rat brain homogenates fortified with NADPH caused a decrease in 5-hydroxytryptophol with a simultaneous increase in 5-HIAA formation (Table 4). These results may be explained in part by the presence of a NADPH-linked alcohol dehydrogenase and a $NADP^+$-linked aldehyde dehydrogenase in the whole homogenate where $NADP^+$ shifts aldehyde metabolism towards 5-HIAA formation and away from the NADPH-induced 5-hydroxytryptophol formation. These results also may be explained in part by a $NADP^+$-induced inhibition of the NADPH-linked alcohol dehydrogenase resulting in decreased 5-hydroxytryptophol formation and increased 5-hydroxy-indoleacetaldehyde and 5-HIAA formation. Whether the alcohol dehydrogenase is truly reversible as suggested (Eccleston, Moir, Reading & Ritchie, 1966) can be determined only on the isolated and purified enzyme using both 5-hydroxytryptophol and 5-hydroxyindoleacetaldehyde as substrates.

Eccleston, Moir, Reading & Ritchie (1966) have also suggested that the NADPH-linked alcohol dehydrogenase which they found in rat brain is distinct from the NADH-linked alcohol dehydrogenase found in rat liver. On the basis of the evidence now available (Tables 1 and 2), this conclusion may be correct. They also reported the presence of a NADPH-linked alcohol dehydrogenase in human brain and we have confirmed this for the human hypothalamus, thalamus, caudate nucleus and cerebral cortex (Feldstein, Williamson & Tedeschi, unpublished). 5-Hydroxytryptophol formation was stimulated to a greater extent by NADPH than by NADH in rat (Feldstein & Williamson, 1968 ; Otani, Farrell & Creaven, 1968) and human (Feldstein, Williamson & Tedeschi, unpublished) pineal homogenates.

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