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TRYPTOPHAN METABOLISM IN EXPERIMENTAL PORPHYRIA*

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Abstract—Although there seems to be no qualitative difference in the excretion of certain urinary metabolites of L-tryptophan between normal and porphyric rats, quantitative determination showed that there is a decrease in the level of excretion of L-kynurenine and 3-OH-kynurenine. At the same time, tryptophan pyrrolase activity is diminished in porphyric animals. This is interpreted as being the result, at least in part, of a competitive inhibition between the porphyrins accumulated in the liver during the state of porphyria and the tryptophan pyrrolase heme coenzyme, for the tryptophan pyrrolase apoenzyme.

STUDIES by Price *et al.*[1] on human porphyria showed an increased urinary excretion of kynurenine, 3-OH-kynurenine, 2nd kynurenic and xanthurenic acids. Previous results by Greengard and Feigelson [2–4] demonstrated that tryptophan pyrrolase, the enzyme responsible for the conversion of L-tryptophan to formyl-L-kynurenine, is activated by a heme-type coenzyme. It was also found that this activity is competitively inhibited by both protporphyrin 9 and mesoporphyrin 9.

It has been shown that in experimentally induced hepatic porphyria[5–7] there is an increase in the concentration of free prophyrins in the liver. These facts suggest a possible relationship between the state of porphyria and the abnormal metabolism of tryptophan detected in this case. In this report we present data which demonstrate decreased levels of urinary excretion of kynurenine and 3-OH-kynurenine in experimental porphyria, and at the same time shown the possible connection between these levels and the tryptophan pyrrolase activity detected in these animals.

EXPERIMENTAL

Male albino rats weighing 200–250 g each were used. Sedormid‡ (450 mg/kg body wt.) was given orally once a day in an aqueous suspension. The animals were considered porphyric after two days of urinary porphobilinogen excretion. Seven to nine days of treatment usually produced this condition and the presence of urinary prophobilinogen was determined by the Watson-Schwartz test.[8]

After partial purification of the urine according to the procedure of Synge and Tiselius,[9] qualitative studies of some of the tryptophan metabolites were carried out by

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‡ Allyl-isopropyl-acetyl-urea.

the chromatographic method of Mason and Berg[10] and Diamantstein and Erhart.[11] Quantitative determinations of urinary kynurenine and 3-OH-kynurenine were performed according to the procedure described by Brown and Price[12] and Brown.[13]

L-Tryptophan (800 mg/kg body wt.) was also administered orally in an aqueous suspension. Tryptophan pyrrolase activity was determined in liver homogenates by the method of Knox and Mehler[14] as modified by Greengard and Feigelson.[3]

RESULTS AND DISCUSSION

Chromatographic studies of the urine of normal and porphyric rats show no qualitative difference in the excretion of kynurenine, 3-OH-kynurenine, and kynurenic and xanthurenic acids. However, quantitative determinations showed that in experimentally induced porphyria there is a decrease in the urinary excretion of both kynurenine and 3-OH-kynurenine. These results may be seen in Table 1. It is also possible to see that

TABLE 1. QUANTITATIVE DETERMINATION OF URINARY KYNURENINE AND 3-OH-KYNURENINE*

Number of animals		Supplementation	Ky/24 hr (μmole)	Ky/100 ml urine (μmole)	HKy/24 hr (μmole)	HKy/100 ml urine (μmole)
C	30	B	0·56	7·00		
P	24	B	0·37	6·40		
C	20	A	1·73	15·00		
P	22	A	0·74	10·60		
C	26	B			0·46	4·30
P	32	B			0·36	6·30
C	20	A			2·68	22·80
P	20	A			0·98	12·20

* C, normal animals used as control; P, porphyric animals; A, after receiving a single dose of L-tryptophan; B, before receiving a single dose of L-tryptophan; Ky, Kynurenine; HKy, 3-OH-kynurenine. The figures shown are the average values for the number of animals shown in the first column.

after a single dose of L-tryptophan, the per cent increase in the kynurenine and 3-OH-kynurenine excreted in 24 hr is less in porphyric than in normal animals.

The data shown in Table 1 indicate the presence of an abnormal metabolism of tryptophan in Sedormid-induced experimental prophyria. This abnormality could be explained at least in part by a decrease in the activity of tryptophan pyrrolase due to the fact that this enzyme has a porphyrin moiety that acts as coenzyme.

Fig. 1 shows the tryptophan pyrrolase activity in normal and porphyric animals before and after a single dose of L-tryptophan. This experiment demonstrates that less activity is detectable in the liver homogenates of porphyric animals. However, there seems to be little difference in the per cent activation produced by a single dose of L-tryptophan.

It may be that the lower levels of excretion of kynurenine and 3-OH-kynurenine in porphyric animals (Table 1) is due at least in part to a decreased activity of tryptophan pyrrolase in the liver of the intoxicated animals. This possibility is confirmed by the results shown in Fig. 1.

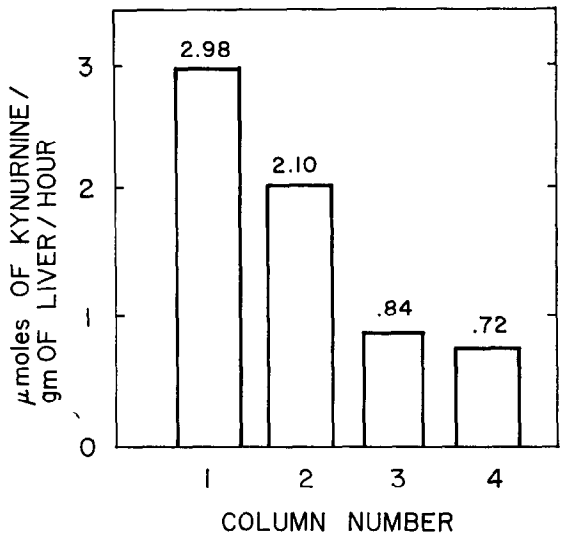

FIG. 1. Determination of tryptophan pyrrolase activity in normal animals during experimental porphyria. Column 1, tryptophan pyrrolase activity in normal animals given a single dose of L-tryptophan 2 hr before being killed; column 2, tryptophan pyrrolase activity in normal animals; column 3, tryptophan pyrrolase activity in Sedormid-treated animals given a single dose of tryptophan 2 hr before being killed; column 4, tryptophan pyrrolase activity in Sedormid-treated animals.

In all cases the incubation mixture contained: phosphate buffer, pH 7·2, 300 μmole; L-tryptophan, 15 μmole; 1·5 ml of the supernatant fraction obtained after centrifugation of the liver homogenates for 30 min at 15,000 g; hematin, 30 μmole; final vol., 3·0 ml. Each column represents the average of 6 different experiments using 3 animals in each experience.

The present results do not allow us to decide whether this decrease in activity is due to a decrease in the saturation of the apoenzyme with its coenzyme, or to a decrease in synthesis, or to an increase in the degradation, of the apoenzyme.

We propose that, at least in part, the effect of Sedormid is due to the accumulation of free porphyrins in the liver. These prophyrins would then exert a competitive inhibition on the saturation of the apoenzyme by its heme coenzyme.

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