CORRELATION BETWEEN TRYPTOPHAN METABOLISM AND THE STATE OF MELANINOGENESIS

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A study of the excretion of tryptophan metabolites and activity of liver tryptophan pyrrolase in black and white rabbits showed that the levels of kynurenin, kynurenic and xanthurenic acids, 3-hydroxykynurenin, anthranilic acid, and 5-hydroxyindoleacetic acid determined in the original urine before L-tryptophan loading differed in the two groups. No 3-hydroxyanthranilic acid was found in the original urine of the rabbits. After administration of L-tryptophan to albino rabbits, a sharp increase was observed in the excretion of all tryptophan metabolites investigated, but in black rabbits there was a sharper increase in the excretion of kynurenic and xanthurenic acids. The liver tryptophan pyrrolase reacts more intensively in white than in black rabbits to administration of L-tryptophan.

KEY WORDS: tryptophan; melaninogenesis; tryptophan metabolites; tryptophan pyrrolase.

According to the generally accepted scheme of melaninogenesis, melanins of animal origin are synthesized from tyrosine [4, 6, 12]. However, besides tyrosine, another chromogenic amino acid is tryptophan. The black pigment obtained from tryptophan, kynurenin, and 3-hydroxykynurenin by the action of tyrosinase is very similar in its properties to sepia- and tyrosinomelanin; the melanin-like substances isolated from human urine have been shown to have a regulatory effect on the activity of kynureninase, kynurenin transaminase, and tryptophan pyrrolase [11]. Melatonin, a metabolite of tryptophan, is known to have the property of decolorizing frogs' skin [5].

In this paper the results of an investigation of tryptophan metabolism in animals differing in their degree of melaninogenesis are described.

EXPERIMENTAL METHOD

Experiments were carried out on 48 white and black rabbits weighing 2.0-3.1 kg, during the summer months (July and August). All the animals were kept under identical laboratory conditions on similar diets. 5-Hydroxyindoleacetic acid (5-HIAA) was determined in the 24-h sample of urine [1]. The diurnal excretion of kynurenin, 3-hydroxykynurenin, anthranilic and 3-hydroxyanthranilic acids, and kynurenic and xanthurenic acids was determined by two-dimensional paper chromatography, using a mixture of butanol, acetic acid, and water (4:1:5, top layer) as the solvent in one direction, and distilled water in the other direction [7, 8, 10]. Tryptophan pyrrolase activity [13], calculated per milligram protein in the incubation medium, was determined by Lowry's method [9]. The following preparations of tryptophan were used: L-tryptophan and anthranilic acid from Reanal, 5-HIAA and xanthurenic acid from Fluka, and kynurenic acid from Austrowaren. The kynurenin, 3-hydroxykynurenin, and 3-hydroxyanthranilic acid were generously provided by Dr. Biol. Sci. E. V. Goryachenkova, to whom the writers are grateful.

EXPERIMENTAL RESULTS AND DISCUSSION

Loading the animals with tryptophan led to an increase in the excretion of many of its metabolites, and with an increase in the substrate loading, the excretion of tryptophan metabolites with the urine rose more sharply [5, 14]. By prolonged (for 1 week) loading of the animals with L-tyrosine, latent differences in

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TABLE 1. Dynamics of Diurnal Excretion of Tryptophan Metabolites (in mg/day) of White and Black Rabbits (5 animals of each color) during Repeated Administration of L-Tryptophan

Tryptophan metabolites	Rabbits	Initial level	After loading with L-tryptophan, mmoles/kg					
			0,5	1	2	3	—	—
			1st day	2nd day	3rd day	4th day	5th day	6th day
Kynurenin	White	—	3,60±2,57	3,60±1,80	4,98±1,78	7,81±2,47	—	—
	Black	1,16±0,66	1,56±0,95	3,61±1,53	4,65±2,69	2,58±0,82	0,22±0,13	Traces
Kynurenic acid	White	3,16±0,87	33,09±5,45	57,38±7,42	105,03±12,44	116,62±18,54	5,83±3,07	10,33±2,96
	Black	7,93±1,91	50,82±4,24	75,62±9,49	107,31±32,87	173,07±49,70	14,72±3,96	14,33±4,32
3-Hydroxykynurenin	White	1,30±0,99	8,94±5,35	14,39±4,24	20,71±15,32	19,62±13,99	Traces	Traces
	Black	1,46±0,78	5,94±2,39	10,41±3,02	11,15±3,16	8,91±5,72	3,55±1,85	5,98±4,60
Xanthurenic acid	White	0,38±0,18	1,73±0,54	3,80±1,02	8,55±2,83	9,15±2,29	0,55±0,35	1,20±0,47
	Black	1,54±0,81	7,11±2,15	13,86±2,66	19,38±5,72	25,72±10,84	1,27±0,47	1,09±0,42
3-Hydroxyanthranilic acid	White	—	Traces	Traces	6,25±3,67	5,93±3,19	—	—
	Black	—	—	—	—	5,90±3,41	—	—
Anthranilic acid	White	—	0,79±0,66	2,78±1,21	10,88±4,34	23,29±5,16	—	—
	Black	1,86±1,14	6,01±3,31	6,90±3,47	6,00±4,11	10,20±6,41	Traces	Traces
5-HIAA	White	0,87±0,16	1,29±0,32	1,13±0,18	1,33±0,27	1,38±0,24	0,73±0,44	0,88±0,19
	Black	0,93±0,23	0,96±0,10	1,10±0,20	1,19±0,31	0,98±0,30	0,78±0,19	0,99±0,45

Legend. Values given are M ± m.

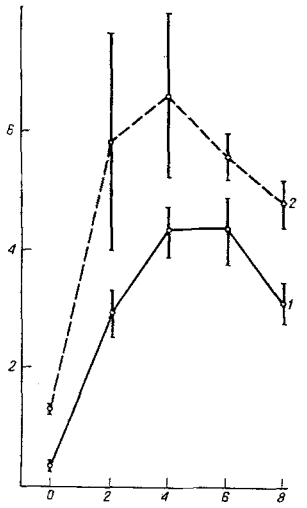

Fig. 1. Dynamics of activity of liver tryptophan pyrrolase in black (1) and white (2) rabbits after a single dose of L-tryptophan by the intragastric route (2 mmoles/kg). Initial level – mean value of enzyme activity in livers of 3 rabbits. Each point after loading represents mean value of 4 experiments. Abscissa, time (in h) after tryptophan loading; ordinate, enzyme activity (in μg kynurenin/mg protein/h).

tyrosine metabolism were discovered previously by the writers in animals with different degrees of melaninogenesis [3].

It was accordingly decided to investigate the dynamics of excretion of tryptophan metabolites for a period of 1 week after prolonged and repeated loadings with L-tryptophan. For this purpose, white and black rabbits (five of each color) had initial 24-h samples of urine collected, after which they were given L-tryptophan by gastric tube in a dose of 0.5 mmole/kg body weight during the first day, 1 mmole/kg on the 2nd day, 2 mmole/kg on the third, and 3 mmole/kg on the fourth day.

Urine was collected during the four days of the experiment and the two days after the end of L-tryptophan administration. All the metabolites of tryptophan tested for, except 3-hydroxyanthranilic acid, were found in urine collected from the rabbits before substrate loading (initial sample; Table 1). However, the excretion of the tryptophan metabolites by the white rabbits was lower than by the black rabbits. Whereas kynurenin and anthranilic acid were excreted with the urine of two of the five black rabbits, these compounds were absent from the original urine of the white rabbits. Although kynurenic and xanthurenic acids and 5-HIAA were present in the original urine of nearly all the rabbits, their concentrations were appreciably lower in that of the white rabbits.

After loading of the animals with L-tryptophan the excretion of its metabolites increased, and with an increase in the quantity of substrate administered, the excretion of its metabolites rose more sharply still. During the first day after the end of L-tryptophan loading the blood level of tryptophan metabolites, except kynurenic acid and, in the black rabbits, 3-hydroxykynurenin, returned to normal or actually fell a little below it. Disregarding a few cases, statistically significant differences could not be found in the excretion of individual tryptophan metabolites by the rabbits depending on the state of melaninogenesis. However, over a period of time the excretion of kynurenin, 3-hydroxykynurenin, 3-hydroxyanthranilic and anthranilic acids, and 5-HIAA by the white rabbits was greater than that of the black rabbits; moreover, these compounds were absent from the original urine of the white rabbits or their level was comparatively low; in black rabbits the level of these metabolites in the urine after L-tryptophan loading increased relatively slowly, and after the end of loading it still remained fairly high. Both in the original urine and after substrate loading, the level of kynurenic and, in particular, of xanthurenic acids remained higher in the black rabbits. The additional L-tryptophan administered to the animals of this group was evidently utilized mainly through the transamination of kynurenin and, in particular, of 3-hydroxykynurenin, whereas in white rabbits this process was accompanied by activation of nearly all stages of the kynurenin pathway of tryptophan breakdown.

Analysis of the results thus indicates the existence of marked differences in the enzymic processes involved in tryptophan metabolism in response to loading with this amino acid in white and black rabbits. The same conclusion can also be drawn from the results of the investigation of the dynamics of liver tryptophan pyrrolase activity in white and black rabbits after a single loading (2 mmoles/kg) with L-tryptophan (Fig. 1). Investigations of tryptophan and tyrosine metabolism in patients with pathology of melanin metabolism has shown that vitiligo is accompanied by intensification of the kynurenin and serotonin pathways of tryptophan catabolism and of the oxidative catabolism of tyrosine [2].

It can thus be postulated that tryptophan metabolism follows a different course depending on the initial state of melaninogenesis.

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