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THE ACTIVITIES OF SOME ENZYMES OF TRYPTOPHAN METABOLISM IN FETAL, NEONATAL AND ADULT RAT LIVER AND KIDNEY

II. KYNURENINE 3-HYDROXYLASE ACTIVITY

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(1) In fetal rat liver and kidney, kynurenine 3-hydroxylase activity was absent. It began to appear after birth and reached the adult level when the body weight of rat became about 50 g (approximately 25 days after birth).

(2) In kidney, the change of kynurenine 3-hydroxylase activity after the daily subcutaneous injection of progesterone or estradiol-17 β in a dose of 2.7 mg or 30 μg per 100 g of body weight, respectively, on the back for one and two weeks was almost same, while in liver, the inhibition of its activity was much more enhanced by the treatment for two weeks than one week.

(3) The results of this experiment and the previous report[4] indicate that anthranilic acid, derived from kynurenine by kynureninase, may play a significant role in fetal rat.

Auerbach *et al.*[1] and Nemeth[2] demonstrated that tryptophan pyrrolase activity was absent from fetal liver of rat. It was reported by Auricchio *et al.*[3] that there was no activity of tryptophan pyrrolase nor 3-hydroxyanthranilic acid oxidase in fetal rat liver, and that these enzymes matured after birth at different rates.

But there has been no report on the activities of kynureniase, kynurenine aminotransferase and kynurenine 3-hydroxylase in fetal rat. The present author and his coworkers[4] previously reported the following facts; in fetal rat, kynureninase activity was present, but kynurenine aminotransferase activity was absent. The activity of the latter arose after birth and reached the adult level at about 50 g of body weight (approximately 25 days after birth). Furthermore, they found that the activities of these two enzymes were remarkably inhibited by female hormones, especially estradiol-17 β *in vivo*. This suggests that those two enzymes of tryptophan metabolism were controled by some hormones during fetal period of rat. In the present research, the author investigated the kynurenine 3-hydroxylase activity in liver or kidney of developing rat, and the effect of progesterone or estradiol-17 β on it.

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MATERIALS AND METHODS

Albino rats during fetal, neonatal and adult life were used in this experiment. Female rats weighing about 100 g were treated with progesterone (2.7 mg per 100 g of body weight) or estradiol-17 β (30 μg per 100 g of body weight) by the subcutaneous injection on the back for one week to one group or for two weeks to another.

NADP and glucose-6-phosphate were purchased from Sigma Chemical Company. L-kynurenine sulfate monohydrate was obtained from California Corporation for Biochemical Research. L-3-hydroxykynurenine was a gift from Senju Pharmaceutical Co., Osaka. Progesterone and estradiol-17 β were obtained from Teikoku Zoki Pharmaceutical Co., Tokyo. Glucose-6-phosphate dehydrogenase was prepared from baker's yeast according to the method of Kornberg *et al.*[5]. All other reagents were of reagent grade.

Preparation of Mitochondria

Rats were killed by exsanguination after they were stunned by a blow on the head. The liver and the kidney were removed and chilled immediately by immersing in ice-cold solution of 0.25 M saccharose. The liver and the kidney mitochondria were prepared according to the method described in the previous report[4].

Assay of Enzyme Activity (Modification of Inagami's Method)[6]

The incubation mixture contained 50 μ moles of Tris-HCl buffer, pH 8.2, 20 μ moles of nicotinamide, 10 μ moles of potassium chloride, 10 μ moles of potassium cyanide, 0.3 μ mole of NADP, 2 μ moles of glucose-6-phosphate, 0.6 unit* of glucose-6-phosphate dehydrogenase, 1/3 μ mole of L-kynurenine sulfate, and an aliquot of an enzyme preparation. Final volume of incubation mixture was 1.0 ml. The mixture was incubated at 37°C for 10 min. After the reaction was stopped by adding 1.0 ml of 10% trichloroacetic acid, the incubation mixture was deproteinized by centrifugation. 1.2 ml of the deproteinized reaction mixture was taken out and its optical density was measured spectrophotometrically at 400 mμ, and then, after adding 0.2 ml of 0.1% sodium nitrite solution to it, the optical density was again measured at 400 mμ. The amount of 3-hydroxykynurenine produced was calculated from the difference between the former and the latter. Blank tubes contained all materials except $NADPH_2$-generating system. In all cases, the activity of the enzyme was proportional

* Glucose-6-phosphate dehydrogenase activity was assayed by the following method; to 2.7 ml of water in a quartz cell having a light path of 1 cm, 0.1 ml of 0.003 M NADP and 0.01 ml of the enzyme preparation, dissolved in 0.5 M Tris-HCl buffer, pH 8.2, were added. After addition of 0.1 ml of 0.02 M glucose-6-phosphate, the optical density at 340 mμ was recorded at 1 minute intervals. One unit of enzyme activity was defined as the amount which caused an initial change in optical density of 1.000 per minute at room temperature (20°C).

to the amount of tissue added and increased linearly with time for at least 15 min. The enzyme activity was expressed as micromole of 3-hydroxykynurenine formed per hour per mg of tissue preparation. The enzyme protein was determined by the biuret and dry weight methods.

RESULTS AND DISCUSSION

The activity of kynurenine 3-hydroxylase in liver or kidney of developing rat was shown in Fig. 1 and Fig. 2.

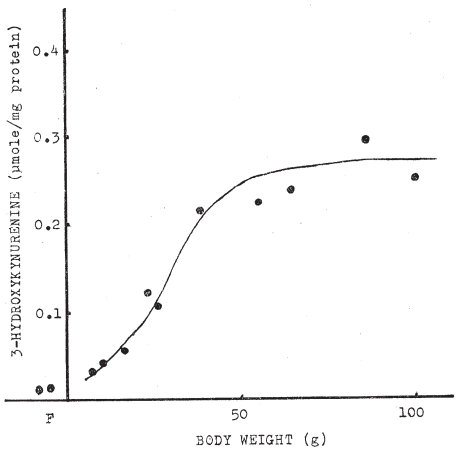

FIG. 1

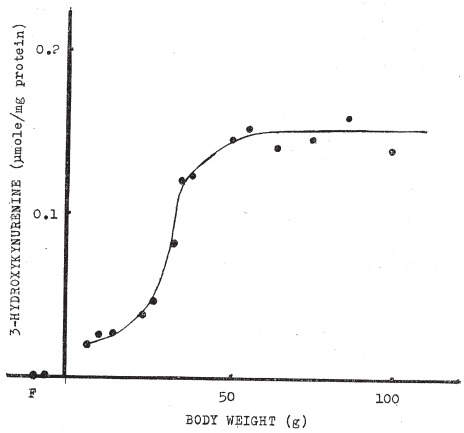

FIG. 2

FIG. 1. Activity of kynurenine 3-hydroxylase in fetal, neonatal and adult rat liver as a function of body weight. The enzyme was assayed as described under "MATERIALS AND METHODS".

F: Activity of kynurenine 3-hydroxylase in fetal rat liver at the 19th-21st day of intrauterine life.

FIG. 2. Activity of kynurenine 3-hydroxylase in fetal, neonatal and adult rat kidney as a function of body weight. The enzyme was assayed as described under "MATERIALS AND METHODS".

F: Activity of kynurenine 3-hydroxylase in fetal rat kidney at the 19th-21st day of intrauterine life.

There was little activity in liver of fetal period. But, in kidney, the activity was absent. After birth, this enzyme activity increased and reached the adult level 25 days after birth (body weight, 50 g). The change of activity of this enzyme was the same as that of kynurenine aminotransferase activity, but much different from that of kynureninase activity, as described in the previous report[4]. The low activity of kynurenine 3-hydroxylase from the fetal period to the neonatal period indicates that this enzyme is not synthesized in that

period (Figs. 1 and 2), or some hormones, especially female hormones, may inhibit the synthesis of this enzyme during that period. The effect of progesterone or estradiol-17 β on the kynurenine 3-hydroxylase activity was shown in Figs. 3 and 4.

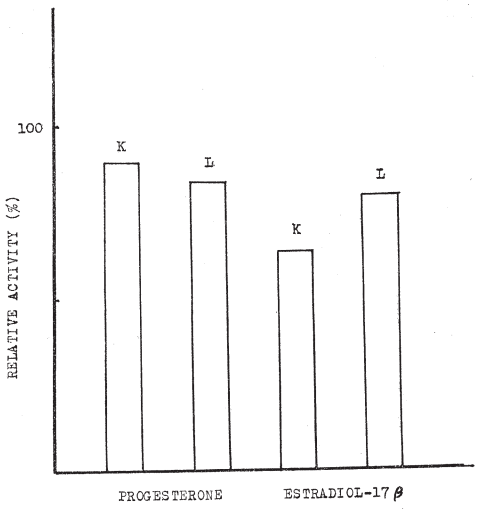

FIG. 3

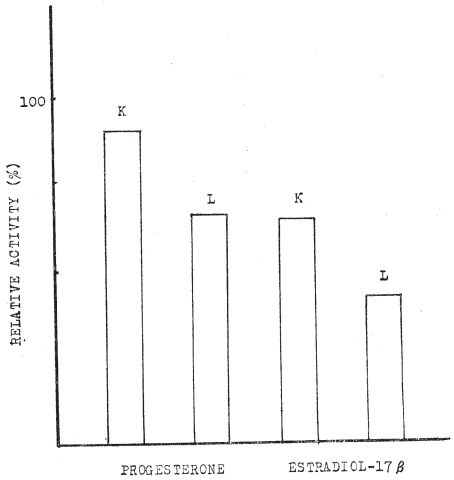

FIG. 4

FIG. 3. Activity of kynurenine 3-hydroxylase after administration of progesterone or estradiol-17 β for one week. The figure shows the percentages of the specific activities in hormone-treated rats to those in the control.

Female albino rats weighing about 100 g were used in this experiment.

L: Liver, K: Kidney

FIG. 4. Activity of kynurenine 3-hydroxylase after administration of progesterone or estradiol-17 β for two weeks. The figure shows the percentages of the specific activities in hormone-treated rats to those in the control.

Female albino rats weighing about 100 g were used in this experiment.

L: Liver, K: Kidney

The activity of kynurenine 3-hydroxylase in kidney was not so much inhibited by progesterone, while it was remarkably inhibited by estradiol-17 β. But, on the kynurenine 3-hydroxylase activity in liver, the injection of progesterone or estradiol-17 β for one week had a slight effect, and the injection for two weeks gave considerable effect on the activity. From the above mentioned results and the previous report[4], it is suggested that anthranilic acid, derived from kynurenine by kynureninase, plays a certain role which may be physiologically significant in fetal rat, and that some hormones, especially female hormones, also control the activities of these enzymes of tryptophan metabolism.

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