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ESTROGEN INDUCTION OF ORNITHINE AMINOTRANSFERASE IN RAT KIDNEY SLICES

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Summary

Immunologic titration of kidney extracts from control and estradiol-injected rats with anti-ornithine aminotransferase serum shows that the hormone induces the enzyme by increasing its concentration in the kidney. The induction has been studied in vitro with kidney slices. Several-fold increase in the rate of ^{14}C-leucine incorporation into the enzyme by hexestrol has been demonstrated. In addition, certain antiestrogens also induce the enzyme synthesis in vitro. These results show that the induction of this enzyme in the kidney does not require the participation of an estrogen metabolite from another tissue.

Introduction

A previous report from this laboratory showed that, in addition to 17β-estradiol, a number of estrogens, such as mestranol, diethylstilbestrol, and hexestrol, could also increase ornithine aminotransferase (OAT) (EC 2.6.1.13) activity in rat kidney in vivo, although 17α-estradiol was without activity (1). These observations have raised two pertinent questions. First, does the increase in OAT activity represent an induction of enzyme synthesis? Second, is a hepatic metabolite of the estrogen required for the observed increase? The experiments described in this communication provide answers to these two questions.

Materials and Methods

Purification of OAT from rat kidney-- Kidneys from young adult female Sprague-Dawley rats were used to obtain the purified enzyme. Purification of OAT from rat kidney followed the general procedure for purifying the enzyme from rat liver (2) with slight modifications. The purified kidney enzyme had a specific activity of 793 μmoles/ mg protein/ hr, compared to 650 μmoles/ mg protein/ hr for the purified rat liver OAT (3). It showed a single band in SDS-polyacrylamide gel electrophoresis as determined with the procedure of Weber and Osborn (4). The subunit weight estimated from these experiments gave a value of 52,000, which agrees well with that obtained for rat liver OAT (3).

Antibody production-- The purified kidney OAT was used to produce anti-

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OAT serum in the New Zealand white rabbit by injecting an emulsion of OAT in Freund's adjuvant into the foot pads (5). The initial injections consisted of 3 mg of OAT. Booster shots containing 2 mg of OAT were given 10 days later. Sera with similar titers were obtained between 7 and 50 days after the booster shots. They were used without further treatment for the experiments described herein.

Animals-- Mature, ovariectomized Sprague-Dawley rats were used at least 2 weeks after surgery by decapitation under light anesthesia. The kidneys were removed and sliced to a thickness of 1 mm. When estrogen-injected rats were used in some experiments, the procedure used before (1) was followed.

Immunologic titration-- We prepared two kinds of supernatant fluids (20,000 x g, 10 min), one from control animals and the other from estradiol-injected animals. Different aliquots (0.01 - 0.40 ml) of each supernatant fluid were taken to react with 10 µl of the anti-OAT serum or a control serum in the presence of 0.9% NaCl and brought to 1 ml with 0.01 M phosphate buffer, pH 7.4. After incubation at 4° for 24 hr, the tubes were centrifuged at 10,000 x g for 10 min. Aliquots of the supernatant fluids were assayed for OAT activity.

Incubation of kidney slices-- About 400 mg of kidney slices were incubated in 2 ml of Eagle's HeLa medium (Difco) containing 5.0 µCi of 2-^{14}C-L-leucine (52 mCi/ mmole, Schwarz/Mann) and 20 µl of test compound or ethanol (for control). The concentrations of the test compounds are shown in the table. The incubation was done in 25-ml conical flasks under an atmosphere of 95% O_2 - 5% CO_2 with constant shaking at 37° for 6 hr. At end of the incubation, the kidney slices were rinsed briefly twice in ice-cold 0.01 M phosphate buffer, pH 7.4. They were then homogenized in 1.5 ml of 0.2 M KCl - 0.01 M phosphate buffer, pH 7.4, containing 0.2% Na dodecyl sulfate to solubilize the mitochondrial OAT. The homogenate was centrifuged at 20,000 x g for 10 min at 4°. The supernatant fluid was used for immunologic determinations.

Immunochemical assay-- Into a series of test tubes, 200 µl of the supernatant fluid obtained above, 50 µl of 9% NaCl, 200 µl of 0.01 M phosphate buffer, pH 7.4, and 50 µl of the anti-OAT serum or a control serum were added. The contents of the tubes were thoroughly mixed and kept at 4° for 20-24 hr. The precipitates in the tubes were collected on microporous filters (0.45 µm, 25 mm diameter, Amicon) and washed with several ml of cold 0.9% NaCl. Each filter was then dissolved in 1 ml of 2-methoxyethanol in a counting vial, followed by 10 ml of a scintillation fluid (6.4 g PPO in 800 ml toluene and 200 ml 2-methoxyethanol). The counting was done in a Packard Tri-Carb Model 3310 with 85% efficiency.

Total proteins in the 20,000 x g - supernatant fluids from kidney slices were precipitated in 3.3% trichloroacetic acid, collected on microporous filters, and washed with the acid. The counts were determined as for the labeled OAT.

OAT activity assay-- The enzyme activity was assayed according to Herzfeld and Knox (6), but with the concentration of α-ketoglutarate increased to 0.02 M in the assay medium. One unit of activity is defined as the amount of enzyme that will cause the formation of 1 µmole of glutamate γ-semialdehyde per hr. Protein was determined by the procedure of Lowry et al. (7).

Results and Discussion

To determine whether estrogens induce OAT in rat kidney in vivo, we used

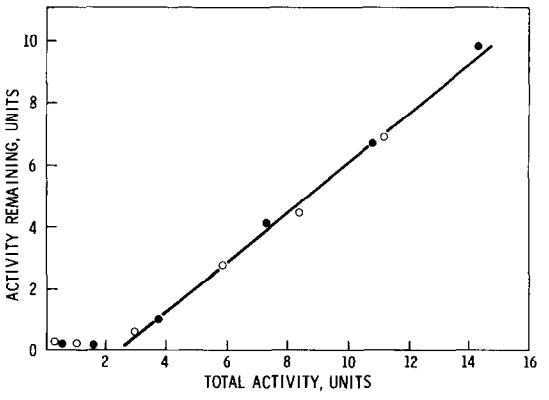

Fig. 1 Immunologic titration of kidney extracts from control (O) and estradiol-injected (●) ovariectomized rats with an anti-OAT serum. OAT specific activity in the control extract was 2.5 units/ mg protein and that in the estradiol-injected extract was 14.7 units/ mg protein. "Total activity" is OAT activity after incubating with a control serum and "activity remaining" is OAT activity after incubating with the anti-OAT serum. For details, see text.

two supernatant fluids: one from a control rat kidney with OAT specific activity of 2.6 units/ mg protein, and the other from an estradiol-injected rat kidney with OAT specific activity of 14.7 units/ mg protein. Immunologic titration of these supernatant fluids with control and anti-OAT sera was done as described earlier. Fig. 1 shows the results of the titration. We can see that the two curves coincided and they had the same equivalence point and slope. Hence, the 6-fold increase in the specific activity after estradiol injection is reflected by a corresponding increase in the amount of OAT in the kidney. This is a clear indication that the hormone induces the enzyme in rat kidney in vivo.

To determine whether a metabolite of the estrogen from another tissue such as liver is the real inducer of OAT in kidney, we studied the induction in kidney slices. Table 1 summarizes the results, which are expressed as a ratio of cpm in OAT x 100 to cpm/ mg protein in the supernatant fluid, so that variance in metabolic activity from different kidneys is minimized. We can see that 17β-estradiol and hexestrol increased the rate of incorporation of ^{14}C-leucine into OAT. With 10^{-4} M hexestrol, the increase was about 4-fold. The induction, however, required relatively high concentrations of the estrogen and a fairly long period of incubation time- 6 hr. Ip et al. (3) have determined the half life of OAT in rat liver to be about 1.9 days, indicating that the rate of

Table 1 Incorporation of ^{14}C-leucine into OAT by estrogens and antiestrogens in rat kidney slices

Compound added	$\frac{\text{cpm OAT} \times 100}{\text{cpm/ mg protein}}$ *
None	1.21
17β-Estradiol, 2×10^{-5} M	1.95
Estrone, 10^{-4} M	0.59
Hexestrol, 2×10^{-5} M	1.11
5×10^{-5} M	3.26
10^{-4} M	4.33
Nitromifene, 5×10^{-4} M	1.51
Enclomiphene, 5×10^{-4} M	2.59
Nafoxidine, 5×10^{-4} M	5.29
Nafoxidine, 5×10^{-4} M + hexestrol, 5×10^{-5} M	9.61

*Each value is the average of 2 to 8 determinations from 1 to 4 experiments.

OAT synthesis is low. If this is also true of the kidney enzyme, it probably explains the need for a rather long period of incubation time to get significant incorporation of ^{14}C-leucine into the enzyme. The requirement for high concentrations of estrogen in the incubation medium presumably results from low concentrations of estrogen receptor in the kidney (8) and the ease with which estrogen is lost from this tissue (9). The experiments done earlier in vivo (1) also showed that the increase in OAT activity in rat kidney occurred at several hours after a relatively large dose of estrogen. On the other hand, estrone was found to be ineffective as an inducer.

Furthermore, we also studied the induction with a number of antiestrogens. Compared at 5×10^{-4} M, nafoxidine hydrochloride (U-11,100A) was the most effective, followed by enclomiphene citrate (formerly "cisclomiphene"), but nitromifene citrate (CI-628) seemed to have little activity. When both hexestrol and nafoxidine were added into the incubation medium, the increase in OAT synthesis rate was essentially equal to the sum of the two taken separately. Katzenellenbogen and Katzenellenbogen (10) have shown that, at 5×10^{-5} M, U-11,100A was partly active and CI-628 was inactive in inducing a specific

protein synthesis in vitro in the uterus of the rat.

We have demonstrated in these experiments that estrogen induction of OAT can take place in rat kidney slices and we conclude that a hepatic metabolite of the hormone is not involved in the inductive process.

Acknowledgments

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