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INDUCTION OF A DECREASE IN RENAL NAD$^+$-DEPENDENT 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE ACTIVITY BY ESTRADIOL IN RATS

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Abstract—The effect of estradiol administration on renal and pulmonary NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase activities in rats was studied. Estradiol induced a significant decrease in renal but not in pulmonary enzyme activity. Kinetic parameters for the renal enzyme from control and treated groups were compared. An identical apparent K_m for prostaglandin E_2 was obtained for the enzyme from both groups. V_{max} in the treated group was progressively decreased and plateaued 1 day after estradiol injection. The estradiol-induced decrease in renal enzyme activity was blocked by an anti-estrogen, nafoxidine, suggesting that the effect of estradiol was a receptor-mediated event. The decrease in renal prostaglandin catabolic enzyme activity induced by estradiol may result in prolonging the half-life of circulating prostacyclin and may account, in part, for the anti-thrombogenic effect of estradiol.

We have been interested in systematically studying the effects of estradiol on the arachidonate cascade at the blood-vessel interface, since epidemiologic studies indicate that females enjoy a lower incidence of coronary artery diseases throughout their reproductive years than males of comparable age [1]. Using rats as an experimental model, we have found that estradiol inhibits the formation of arterial thrombosis induced by electric shock [2]. A plausible biochemical explanation for the anti-thrombogenic effect of estradiol was provided by the finding that estradiol stimulated selectively prostacyclin synthesis in vascular wall, but not thromboxane formation in platelets [2, 3]. The increase in prostacyclin synthesis was attributed to stimulation of *de novo* synthesis of fatty acid cyclooxygenase in vascular cells [4–6].

It has been suggested that the circulating prostacyclin is primarily inactivated by NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase [7]. This enzyme has been shown to be enriched in kidneys and lungs [8]. Alteration of the enzyme activity by any means may influence the physiological functions of these organs. We have determined the effect of estradiol administration on renal and pulmonary enzyme activities in rats. Our results indicate that estradiol significantly decreased renal but not pulmonary enzyme activity.

MATERIALS AND METHODS

Chemicals. Bovine liver glutamate dehydrogenase (51 units/mg), α-ketoglutarate monosodium salt, NAD$^+$, 17β-estradiol and bovine serum albumin (fraction V) were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Depo-estradiol cypionate dissolved in cotton seed oil, prostaglandin E_2, 15-ketoprostaglandin E_2 and nafoxidine HCl were obtained from the Upjohn Co., Kalamazoo, MI, U.S.A. 15(S)-[15-^{3}H]Prostaglandin E_2 was prepared according to Tai [8]. All reagents not specified above were of analytical grade.

Animals. Male Sprague–Dawley rats aged 6–8 weeks, with body weights ranging from 220 to 260 g, were provided by Harlan Sprague–Dawley Inc., Indianapolis, IN, U.S.A. Upon receipt, all animals were maintained on a commercial laboratory feed under a constant light–dark cycle (8:00 a.m. to 8:00 p.m.), and water was provided *ad lib.*

Drug treatment. Depo-estradiol cypionate in cotton seed oil was administered subcutaneously. Controls received the same volume of cotton seed oil (1 ml/kg body weight) only. Estradiol was given at a dose of 0.5 mg/kg body weight, and was repeated at intervals of 3–4 days, unless otherwise stated. Nafoxidine HCl, an anti-estrogen, was dissolved in 0.15 M NaCl at a dose of 5 mg/kg body weight, and was injected subcutaneously together with estradiol. Controls received an equal volume of saline vehicle and cotton seed oil.

Preparation of crude enzyme extract. Animals were killed by bleeding from the abdominal aorta under light ethyl ether anesthesia. Kidney and lung were immediately excised and frozen in dry ice. Tissues were homogenized in 5 vol. of 0.05 M Tris–HCl, pH 7.5, using a modified Potter–Elvehjem homogenizer. The homogenates were centrifuged at 27,000 g for 20 min. The supernatant fraction was used as the source of NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase.

Assay of NAD$^+$-dependent hydroxyprostaglandin

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dehydrogenase. Enzyme activity was determined by measuring the transfer of tritium from 15(*S*)-[15-^{3}H]prostaglandin E_2 to glutamate by coupling with glutamate dehydrogenase as described previously [8]. Briefly, the reaction mixture contained: NH_4Cl, 5 μmoles; α-ketoglutarate, 1 μmole; NAD^+, 1 μmole; 15(*S*)-[15-^{3}H]prostaglandin E_2, 1 nmole, 30,000 cpm; glutamate dehydrogenase, 100 μg; and crude enzyme extract in a final volume of 1 ml of 0.05 M Tris–HCl, pH 7.5. The reaction was continued for 10 min at 37° and terminated by the addition of 0.3 ml of 10% aqueous charcoal suspension. The radioactivity in the supernatant fraction after centrifugation (1000 *g*, 5 min) was determined by liquid scintillation counting. Calculation of the amount of prostaglandin E_2 oxidized was based on the assumption that no kinetic isotope effect was involved in the oxidation of the 15(*S*)-hydroxyl group of 15(*S*)-[15-^{3}H]prostaglandin E_2 as a substrate.

Protein determinations. Protein contents were determined by the method of Lowry *et al.* [9] with bovine serum albumin (Fraction V) as a standard.

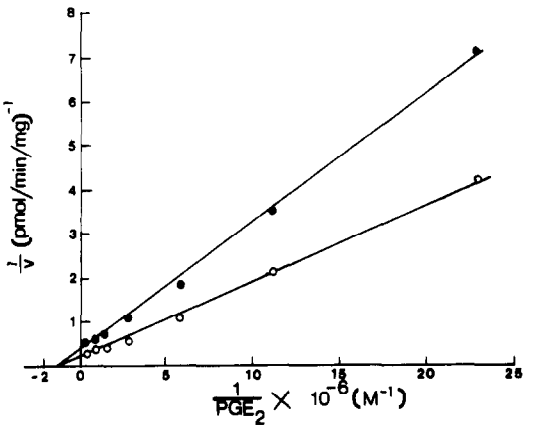

Fig. 1. Effect of substrate concentration on renal 15-hydroxyprostaglandin dehydrogenase activity. Rats were injected at 3- to 4-day intervals with 0.5 mg estradiol/kg body weight for 4 weeks. Open and closed circles represent control and estradiol-treated groups respectively.

RESULTS

Effect of estradiol treatment in rats on renal and pulmonary 15-hydroxyprostaglandin dehydrogenase activities. NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity in both control and estradiol-treated kidneys was linear up to 20 min of incubation. Enzyme activity was also linearly related to protein concentration up to 5 mg/ml (data not shown). Linear range of incubation time and protein concentration was used in all experiments. Table 1 indicates the effect of estradiol treatment on the enzyme activity in kidneys and lungs. Rats were treated with estradiol for 4 weeks, and the enzyme activity in kidneys and lungs of both groups was determined. Estradiol treatment significantly decreased the enzyme activity in kidneys without any significant effect on the enzyme activity in lungs. Kinetic analysis of the enzyme in kidneys of control and treated groups were performed. A Lineweaver–Burk double-reciprocal plot is shown in Fig. 1. An identical apparent K_m of 0.6 μM for substrate prostaglandin E_2 was estimated for the enzyme from control and estradiol-treated groups. However, different V_{max} values were obtained for the enzyme from control (5 pmoles/min/mg) and estradiol-treated

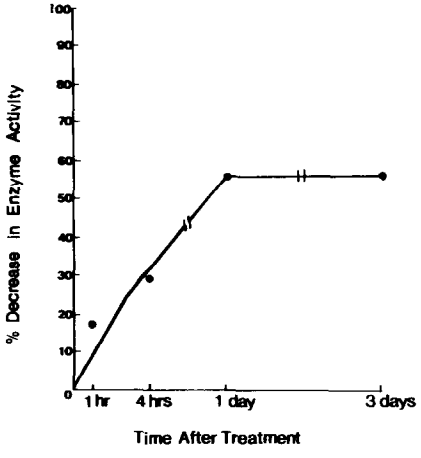

Fig. 2. Effect of length of treatment of estradiol on renal 15-hydroxyprostaglandin dehydrogenase activity. Rats were injected with a single dose of 0.5 mg estradiol/kg body weight. Change in the enzyme activity at each time intervals was compared to their parallel controls. Four rats were used in each group.

Table 1. Effect of estradiol administration on renal and pulmonary 15-hydroxyprostaglandin dehydrogenase activities*

Groups	No. of rats	Enzyme activity [PGE_2 oxidized (pmoles/min/mg)]	
		Kidney	Lung
Control	8	3.0 ± 0.4	2.2 ± 0.3
Estradiol	8	1.3 ± 0.1†	1.9 ± 0.2‡

* Rats were injected at 3- to 4-day intervals with 0.5 mg estradiol/kg body weight for 4 weeks. Kidneys and lungs were removed, homogenized, and assayed for 15-hydroxyprostaglandin dehydrogenase activity as described in Materials and Methods. Values are mean ± S.E.M.

† $P < 0.001$.

‡ Not significantly different from control.

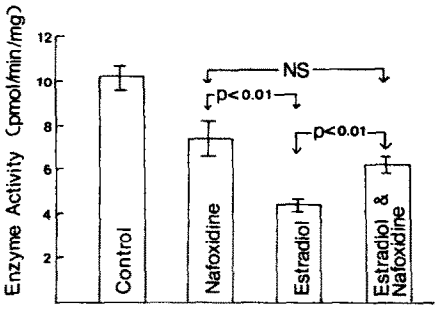

Fig. 3. Effect of the anti-estrogen nafoxidine on estradiol-induced decrease in renal 15-hydroxyprostaglandin dehydrogenase activity. Nafoxidine HCl (5 mg/kg body weight) was injected simultaneously with estradiol (0.5 mg/kg body weight) for 1 day. Five rats were used in each group.

(2.5 pmoles/min/mg) groups. Figure 2 shows the time course of activity decrease following estradiol treatment. The response to estradiol was significantly evident 1 hr after treatment, and the maximal response was reached at 1 day after a single injection of estradiol. Prolonged treatment for 1, 2 and 4 weeks did not show a further decrease in enzyme activity. Direct addition of 10^{-9} to 10^{-3} M estradiol to the crude enzyme extract prepared from control kidneys had no significant effect on the enzyme activity.

Effect of nafoxidine on estradiol-induced decrease in renal 15-hydroxyprostaglandin dehydrogenase activity. Figure 3 shows that simultaneous injection of an anti-estrogen, nafoxidine, and estradiol blocked the estradiol-induced decrease in enzyme activity. Nafoxidine itself caused a 26% decrease in activity as compared to controls whereas estradiol showed a 58% decrease. The effect of estradiol was blocked by nafoxidine.

DISCUSSION

NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase is the first enzyme in the catabolic pathway of prostaglandins. It catalyzes the oxidation of the 15(*S*)-hydroxyl group of virtually all prostaglandins except prostaglandin B_2 [10] and is considered the key enzyme responsible for the biological inactivation of prostaglandins [11]. Changes in this enzyme activity may alter the level of prostaglandins and consequently affect the function of concerned physiological systems.

In the present study, we found that estradiol administration significantly decreased renal 15-hydroxyprostaglandin dehydrogenase activity in rats. The effect of estradiol was relatively rapid and was tissue specific since it did not affect pulmonary enzyme activity. The estradiol-induced decrease in renal enzyme activity could be blocked by nafoxidine. The mode of action of nafoxidine is thought to be due to its ability to reduce the number of estradiol–receptor complexes entering target nuclei [12]. The present finding with nafoxidine indicates that the estradiol-induced decrease in renal enzyme activity is clearly a receptor-mediated event. Although nafoxidine could antagonize the effect of estradiol, it also induced some decrease in enzyme activity by itself. This additional effect of nafoxidine remains unexplained. The enzyme from the estradiol-treated group most likely was not an altered enzyme at the active site since the enzyme from both control and treated groups possessed identical K_m values for substrate prostaglandin E_2. However, specific enzyme activity was decreased, indicating less enzyme was synthesized or more enzyme was degraded after estradiol treatment. Mechanisms that lead to a decrease in enzyme synthesis or an increase in enzyme turnover induced by estradiol remain to be determined.

Although lung is known to be the major organ in inactivating most prostaglandins except prostacyclin and prostaglandin A_2 as they traverse this organ [13], kidney appears to inactivate prostacyclin in one transit [14] and is considered a key organ in metabolizing prostacyclin [15, 16]. Decrease in renal dehydrogenase activity may prolong the half-life of prostacyclin and potentiate the anti-platelet and vasodilatory activities of the prostatcyclin that is generated in the vascular wall. Consequently, the prostacyclin to thromboxane A_2 ratio in circulation, a potential marker for vascular homeostasis, may favor platelet disaggregation and blood vessel dilation.

Uzunova *et al.* [17] have reported that male rats have approximately twice the thrombus size and death rate and a lower obstruction time as compared to females. Estradiol treatment significantly decreased the thrombus weight and increased the obstruction time in male rats. Using an electrically-induced thrombus model in rats, we found that estradiol administration significantly inhibits the thrombus formation induced by electric shock [2]. The present study showing decrease in renal 15-hydroxyprostaglandin dehydrogenase activity suggests that catabolism of prostacyclin *in vivo* might be retarded significantly by estradiol treatment. This finding coupled with our previous reports [3–6] demonstrating selective increase in aortic prostacyclin production by estradiol administration may account, at least in part, for the anti-thrombogenic effect of estradiol.

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