Effect of Vitamin E on Arginase Activity in the Liver and Kidneys of Testosterone-Treated and Castrated Rabbits

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Abstract

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In the present study we examined the effect of vitamin E administration on arginase activity in the liver and kidneys of testosterone-treated and castrated rabbits. Forty-six 3-month-old male rabbits were divided into six groups: 1) control rabbits, 0.5 ml olive oil; 2) testosterone-treated rabbits, 10 mg of testosterone propionate dissolved in 0.5 ml olive oil; 3) bilaterally castrated rabbits, 0.5 ml olive oil; 4) vitamin E-treated rabbits, 100 mg/kg dl-α-tocopheryl acetate dissolved in 0.5 ml olive oil; 5) vitamin E- and testosterone-treated rabbits, 100 mg/kg dl-α-tocopheryl acetate and 10 mg testosterone propionate dissolved in 0.5 ml olive oil; 6) bilaterally castrated and vitamin E-treated rabbits, 100 mg/kg dl-α-tocopheryl acetate dissolved in 0.5 ml olive oil. The administration was done subcutaneously over 24 h for 40 days; then the arginase activities in the liver and kidneys were determined.

Liver arginase activities in all the groups did not change significantly ($p > 0.05$). Kidney arginase activities were not affected by castration and vitamin E. Kidney arginase activity was found to have increased two-fold by testosterone treatment. Testosterone-induced arginase activity in the kidneys returned to normal level with a significant lowering effect of the combination of vitamin E and testosterone.

These results indicate that vitamin E supplementation has a significant reducing effect on the testosterone-induced arginase activity in the kidneys. Vitamin E ameliorates the testosterone-induced arginase activity in the kidneys.

Arginase, testosterone, castration, vitamin E

Arginase catalyzes the hydrolysis of L-arginine to form L-ornithine and urea (L-arginine amidinohydrolase, EC 3.5.3.1). This reaction comprises the final cytosolic step of the urea cycle, which provides the principal route for the disposal of nitrogenous waste from protein catabolism. Although arginase activity is most abundant in the mammalian liver where the urea cycle is most active, it is also found in non-hepatic tissues such as the kidney (Herzfeld and Raper 1976). The kidney does not contain an active urea cycle (Ratner and Petrack 1953), and kidney arginase is probably involved in the catabolism of arginine for use as a source of proline or glutamate, and polyamines (Kaysen and Strecker 1973; Manteuffel-Cymborowska et al. 1993; 1995). Since the liver and kidney arginase types differ in their regulation by certain steroid hormones (Kumar and Kalyankar 1984), by pattern of expression in patients with hyperargininemia (Spector et al. 1983), by electrophoretic mobility and by immunologic relatedness (Skryzpek-Osiecka and Porembska 1983; Porembska and Zamecka 1984; Porembska et al. 1993), they appear to be distinct polypeptides encoded by two different genes (Haggerty et al. 1983).

Vitamin E maintains homeostasis in living cells (Gallo-Torres 1980). Park and Tappel (1991) reported a relationship between vitamin E and arginase: rats fed a vitamin

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E supplemented diet had a lower liver arginase activity than those fed a vitamin E deficient diet. Arginase is known to be inducible by hormones (Mimic-Oka et al. 1971; Yamanaka et al. 1971; Kumar and Kalyankar 1984; Patnaik and Patnaik 1989). This effect can be prevented by vitamin E. Therefore, we examined the effects of vitamin E supplementation on arginase activity in the liver and kidneys of testosterone-treated and castrated rabbits.

Materials and Methods

Animals and Treatments

The experiment was conducted on forty-six 3-month-old clinically healthy adult male New Zealand White rabbits weighing 2600 ± 300 g, which were housed one per cage at room temperature of 20 °C. During a 10-day adaptation period, the animals were given a basic diet and tap water *ad libitum*. At the end of this period, the rabbits were divided into six groups: 1) control rabbits, 0.5 ml olive oil; 2) testosterone-treated rabbits, 10 mg of testosterone propionate dissolved in 0.5 ml olive oil; 3) bilaterally castrated rabbits, 0.5 ml olive oil; 4) vitamin E-treated rabbits, 100 mg/kg dl-α-tocopheryl acetate dissolved in 0.5 ml olive oil; 5) vitamin E- and testosterone-treated rabbits, 100 mg/kg dl-α-tocopheryl acetate and 10 mg testosterone propionate dissolved in 0.5 ml olive oil; 6) bilaterally castrated and vitamin E-treated rabbits, 100 mg/kg d-α-tocopheryl acetate dissolved in 0.5 ml olive oil. This administration was done subcutaneously over 24 h for 40 days.

At the end of the experiment, the rabbits were anesthetized and the liver and kidneys were excised and rinsed in cold saline (0.9 % NaCl). One gram of liver and kidney tissues was weighed and homogenized with 10 volumes of 10 mM Tris-HCl buffer pH (7.4) in a glass Potter Elvehjem homogenizer in an ice bath. The homogenates were centrifuged at 20 000 *g* for 10 min at 4 °C. The supernatants were used for the arginase assay.

Arginase assay

Arginase activity was measured by determining the increase in the amount of the reaction product, urea (Geyer and Dabich 1971). One unit (U) of enzyme activity was defined as μmole of the product formed per hour at 37 °C. The results are given as units/ mg of protein.

Protein determination

The protein content of tissue samples was assayed according to the method of Lowry et al. (1951). The bovine serum albumin was used as the standard.

Statistical Analysis

Results were expressed as mean ± SEM. Analysis of variance (ANOVA) followed by the Duncan test were used to determine significant differences among the groups. A 5% level of significance was used to establish differences.

Results

Liver arginase activities in all the groups did not change significantly ($p > 0.05$). Kidney arginase activities were not affected by castration and vitamin E. No significant differences were found in kidney arginase activity in the combination group compared to the controls ($p > 0.05$). Kidney arginase activity increased two-fold by testosterone treatment. The increase in kidney arginase activity induced by testosterone was reduced by vitamin E supplementation (Table 1).

Table 1. Effect of vitamin E administration on arginase activity (units / mg protein) in the liver and kidneys of testosterone-treated and castrated rabbits

	Control $\bar{x} \pm S_{\bar{x}}$	Testosterone $\bar{x} \pm S_{\bar{x}}$	Castration $\bar{x} \pm S_{\bar{x}}$	Vitamin E $\bar{x} \pm S_{\bar{x}}$	Testosterone + Vitamin E $\bar{x} \pm S_{\bar{x}}$	Castration + Vitamin E $\bar{x} \pm S_{\bar{x}}$	*P*
Liver	299.34 ± 31.23	273.49 ± 13.36	244.72 ± 3.70	267.83 ± 31.82	223.51 ± 10.45	257.52 ± 34.65	NS
	n = 7	n = 7	n = 6	n = 8	n = 8	n = 6	
Kidney	4.33 ± 0.79[a]	8.72 ± 1.29[b]	5.22 ± 0.90[a]	4.47 ± 0.70[a]	5.81 ± 0.94[a]	5.01 ± 0.82[a]	*
	n = 8	n = 9	n = 6	n = 8	n = 8	n = 7	

NS: Not significant

* $p < 0.05$

The differences between the means, marked with different letters (i.e. a, b) within the same line, were statistically significant ($p < 0.05$).

Discussion

Liver and kidney arginases differ in their response to different steroid hormones (Mimic-Oka et al. 1971; Kumar and Kalyankar 1984). It has been established that glucocorticoids increase the activity of liver arginase (Mimic-Oka et al. 1971; Kumar and Kalyankar 1984; Patnaik and Patnaik 1989; Grofte et al. 1998). Different glucocorticoids have different effects on arginase in the kidney. Bilateral adrenalectomy decreased significantly the activity of the kidney cortex arginase in rats, whereas hydrocortisone administered to adrenalectomized rats increased it (Patnaik and Patnaik 1989). Furthermore, renal arginase was much more sensitive than liver arginase to exogenous hydrocortisone; its activity increased by 100% after the injection of small doses of hydrocortisone (Mimic-Oka et al. 1971). On the contrary, Kumar and Kalyankar (1984) did not observe any change in the specific activity of kidney arginase in rats treated with corticosterone.

Kumar and Kalyankar (1984) observed a two-fold increase in the specific activity of kidney arginase in male rats aged 6, 12, 24 and 72 weeks treated with testosterone. Testosterone stimulated kidney arginase, but had no effect on hepatic arginase (Kumar and Kalyankar 1984). Several studies have indicated that administration of testosterone increases kidney arginase activity in rats and mice (Yamanaka et al. 1971; Swank et al. 1977; Kumar and Kalyankar 1984; Manteuffel-Cymborowska et al. 1995); which is due to increased synthesis of the enzyme protein (Frieden and Fishel 1968). We also determined that testosterone treatment induced kidney arginase activity without any effect on the liver enzyme in the male rabbit. It has been found that castration caused no significant changes in arginase activity in the kidneys of rats, and testosterone treatment of castrated rats induced an increase in the enzyme activity (Yamanaka et al. 1971). Furthermore, Swank et al. (1977) found that hypophysectomy in female mice decreased the level of kidney arginase and that this level could be restored in hypophysectomized animals by a treatment with androgens. All these data show that the enzyme is under a regulatory control of androgens.

Park and Tappel (1991) reported that rats fed a vitamin E-supplemented diet for 40 days had a lower liver arginase activity than those fed a vitamin E-deficient diet. In the present study, we observed that vitamin E supplementation significantly reduced the increase in kidney arginase activity caused by testosterone, whereas vitamin E alone had no effect on liver or kidney arginase. We found that a dietary administration of vitamin E also had a significant reducing effect on arginase activity in the liver of rats treated with high doses of prednisolone (Erisir et al. 2003).

In contrast to liver, the kidney ornithine, formed by the action of arginase, is not further metabolized in the urea cycle because of absence of two enzymes of this cycle. Instead, it plays an important role both as a precursor of polyamines, and in the glutamate/proline pathway (Kaysen and Strecker 1973; Manteuffel-Cymborowska et al. 1993; 1995). Administration of testosterone to female mice causes hypertrophy of the kidneys paralleled by a pronounced induction of ornithine decarboxylase which generates putrescine, a substrate for the synthesis of higher polyamines (Manteuffel-Cymborowska 1993; Manteuffel-Cymborowska et al. 1993; 1997). Recent evidence shows that testosterone induces differential changes in the activity of two enzymes involved in ornithine biosynthesis (arginase) and catabolism (ornithine aminotransferase). This is evidenced by sensitive biochemical markers of renal hypertrophy, namely arginase and ornithine aminotransferase, that responded to testosterone treatment with the increase and decrease of activities, respectively (Manteuffel-Cymborowska et al. 1995). It is interesting that renal hypertrophy induced by testosterone was accompanied with an increase of arginase activity. An interesting observation in the present study was that the testosterone-induced

kidney arginase activity was restored to normal level by vitamin E supplementation. We suggest that vitamin E may prevent kidney hypertrophy caused by testosterone, by preventing the increase in arginase activity.

More information is needed, especially corcerning the exact nature of the effect of vitamin E on the testosterone-induced kidney arginase activity.

Vliv vitamínu E na aktivitu arginázy v játrech a ledvinách testosteronem ošetřených a kastrovaných králíků

Aplikace vitamínu E výrazně snižuje aktivitu arginázy v játrech. Argináza je indukována hormony. Tomuto účinku lze předcházet vitamínem E. Proto jsme zkoumali vliv aplikace tohoto vitamínu na aktivitu arginázy v játrech a ledvinách testosteronem ošetřených a kastrovaných králíků.

Pokus byl proveden na 46 dospělých, 3 měsíce starých samcích plemene Novozélanského bílého králíka, vážících 2600 ± 300 g. Králíci byly rozděleni do 6ti skupin; 1. kontrolní králíci, 0,5 ml olivového oleje; 2. testosteronem ošetření králíci, 10 mg testosteronpropionátu (rozpuštěn v 0,5 ml olivového oleje); 3. oboustranně kastrovaní králíci a 0,5 ml olivového oleje; 4. vitamínem E ošetření králíci 100 mg/kg dl-α-tokopheryl acetátu (rozpuštěného v 0.5 ml olivového oleje); 5. vitamínem E a testosteronem ošetření králíci, králíci 100 mg/kg dl-α-tokopheryl acetátu a 10 mg testosteronpropionátu (rozpuštěných v 0.5 ml olivového oleje); 6. oboustranně kastrovaní, vitamínem E a testosteronem ošetření králíci, 100 mg/kg dl-α-tokopheryl acetátu a 10 mg testosteronpropionátu (rozpuštěných v 0.5 ml olivového oleje). Tato aplikace byla provedena subkutánně po 24 hodinách po 40 dní. Na konci pokusu byla zjištěna aktivita arginázy v játrech a ledvinách.

Aktivita arginázy v játrech se v žádné ze skupin významně nezměnila ($P > 0,05$). Aktivita arginázy v ledvinách se nezměnila u kastrovaných a vitamínem E ošetřených králíků. Dvojnásobně zvýšená aktivita arginázy byla zaznamenána u králíků ošetřených testosteronem. U testosteronem vyvolané aktivity arginázy došlo k návratu na normální úroveň za významného redukujícího účinku kombinace vitamínu E a testosteronu.

Tyto výsledky ukazují, že přídavek vitamínu E má signifikantně snižující účinek na testosteronem indukovanou aktivitu arginázy v ledvinách a působí tak na ní pozitivně.

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