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Effect of Androgen and Aging on Ornithine Decarboxylase Activity of Rat Ventral Prostate

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ABSTRACT

The age-related alteration of androgen dependency in ornithine decarboxylase (ODC) activity was examined in ventral prostate from: (1) intact rats aged 6, 10, 50 and 100W; (2) castrated rats aged 10 and 100W; (3) 10 and 100W rats castrated for 7 days before injection with testosterone propionate for up to 7 days. Tissue dihydrotestosterone (DHT) contents in ventral prostate from intact rats aged 10 and 100W were also measured. ODC activity of rat ventral prostate declined with aging. Castration resulted in rapid decline in ODC activity of young mature rats (10W) and the activity was restored to normal value by the injection of testosterone propionate. On the other hand, the changes in ODC activity of old rats (100W) induced by castration and androgen replacement were significantly slower than those of young mature rats. In young mature rats, ODC activity was apparently correlated with prostatic DHT contents. Although prostatic DHT contents of old rats retained a similar level to young mature rats, no such correlation between ODC activity and tissue DHT content was observed.

These results showed androgen-sensitive nature of ODC activity. And the sensitivity might be affected by aging at a step by which androgen showed its action.

Polyamine synthesis has been shown to increased during growth or regeneration in response to the growth-inducing factors, hormones and drugs, in vivo and in cell culture system[3,5]. Ornithine decarboxylase (ODC) is thought to be the rate-limiting factor in the synthetic pathway of polyamine, and ODC activity has been shown to be elevated in the proliferating tissues[8].

ODC activity is very high in the rat ventral prostate that is engaged in active secretion of polyamines. Regulation of ODC activity in the rat ventral prostate by androgen has been reported: castration resulted in rapid decline in ODC activity and the activity was recovered by injection of testosterone in young mature rats[7]. These findings indicated that ODC might be a sensitive and specific parameter of androgen action in the prostate[4].

In the present study, we have examined the effect of androgen and aging on ODC activity of rat ventral prostate and relationship between ODC activity and tissue dihydrotestosterone (DHT) content.

MATERIALS AND METHODS

Chemicals

L-^{14}C-ornithine hydrochloride and Testosterone/Dihydrotestosterone RIA-kit and ACS II liquid scintillation cocktail were obtained from Amersham International plc (Buckinghamshire, England). Testosterone propionate and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). Pyridoxal phosphate, dithiothreitol, disodium ethylendiamine tetraacetate (EDTA) and hyamine hydroxide were obtained from Nakarai Chemical Co. (Kyoto, Japan).

Animals and preparation of cytosol

Wistar rats aged 6, 10, 50 and 100W were used. Castration was performed via scrotal route under ether anesthesia. Testosterone-treated rats received a daily subcutaneous injection of testosterone propionate (1mg) in 0.2 ml of sesame oil. Rats were killed by cervical dislocation and prostate and coagulating gland were immediately dissected out. The dissected tissue was quickly freed from fat and connective tissue and weighed. The weighed tissue was minced and homogenized in 5 volume of ice-cold 10mM sodium phosphate buffer (pH 7.2) containing 2.5mM dithiothreitol and 0.1mM EDTA. A part of homogenate was saved for the determination of DHT content. The remaining homogenate was centrifuged at 105,000g for 1 hr. The resulting supernatant was used for the determination of ODC activity and protein.

Measurement of ODC activity

ODC activity was assayed by the method described by Beavan et al[1]. The reaction was started by mixing 25μl of cytosol with an equal volume of assay buffer in 1.5ml of conical centrifuge tube. The reaction mixture contained (final concentration) 50mM sodium phosphate buffer (pH 7.2), 2.5mM dithiothreitol, 50μM EDTA, 0.4mM pyridoxal phosphate and 2.5mM L-ornithine (2.5mCi/mmol). The centrifuge tube was placed in a 20ml of screw-cap glass liquid scintillation counting vial containing 20μl of hyamine hydroxide. The glass vial was tightly capped and incubated at 37°C for 120 min. At the end of incubation, 15μl of 50% citoric acid was added to the centrifuge tube to terminate the reaction and convert $H^{14}CO_3^-$ to free $^{14}CO_2$. The counting vial was further incubated for 30 min. At the end of second incubation, the centrifuge tube was removed from the counting vial and 6ml of ACS II liquid scintillation cocktail was added to the counting vial. Radioactivity was counted with liquid scintillation counter LSK 700 (Aloka, Japan). All measurements were done in duplicate. The activity was linearly related to the amount of cytosol protein per assay tube through at least 400μg, and to incubation time through at least 120 min.

Others

Protein was determined by the method of Lowry et al[6], using bovine serum albumin as a standard. Prostatic DHT content was measured by Testosterone/Dihydrotestosterone RIA-kit.

RESULTS

ODC activities of different lobes of prostate and coagulating gland in young mature rats(10W) were determined. ODC activity of the ventral prostate was about 10 times and 20 times higher than the dorsal prostate and coagulating gland, respectively (Table). ODC activity was not detected in lateral prostate under the condition used.

Table ODC activity in different lobes of prostate and coagulating gland of young mature rats(10W)

	ODC activity (nmol CO_2/g wet tissue per hr)
Ventral prostate	40.7
Lateral prostate	Not detectable
Dorsal prostate	4.4
Coagulating gland	2.2

ODC activity of the ventral prostate showed age-related decline (Fig. 1). At 50W of age, ventral prostate ODC activity was diminished 65% relative to that of 10W rat. No further decrease in ODC activity was observed with aging from 50W to 100W. There was no significant difference in ventral prostate ODC activity between immature rats(6W) and young mature rats.

Castration resulted in marked decrease in ODC activity of young mature rats. ODC activity of rats castrated for 48 hr was diminished 85% relative to that of untreated young mature rats (Fig. 2). ODC activity became undetectable 7 days after castration. Injection of testosterone

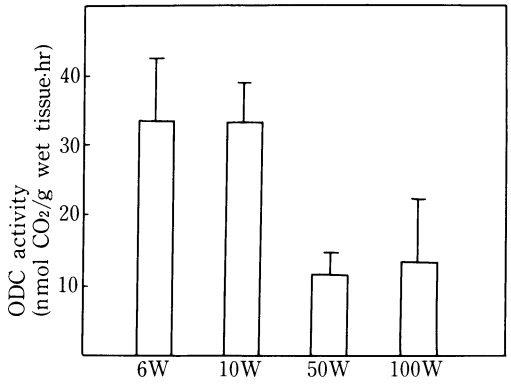

Fig. 1. Age-related change in ventral prostate ODC activity. Each group consisted of 4 rats.

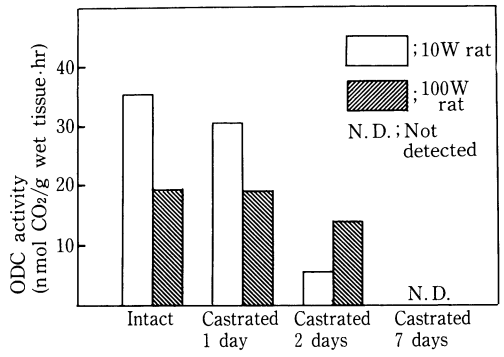

Fig. 2. Effect of castration on ventral prostate ODC activity in young mature rats(10W) and old rats(100W). Each group consisted of 2 rats.

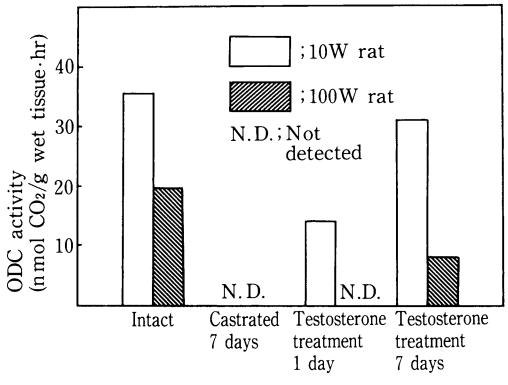

Fig. 3. Effect of testosterone administration on ventral prostate ODC activity in young mature rats and old rats castrated for 7 days. Each group consisted of 2 rats. Rats were given subcutaneous injection of 1mg testosterone propionate in 0.2ml sesame oil per day.

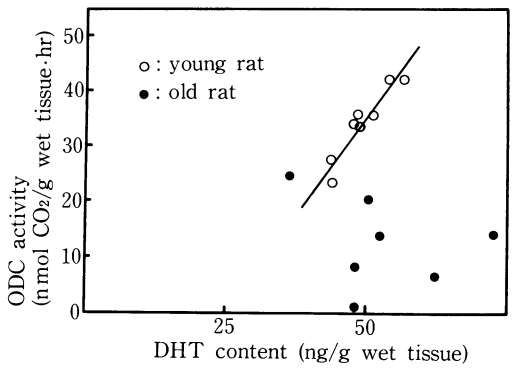

Fig. 4. Relationship between ODC activity and tissue DHT content in young mature rats and old rats. DHT content in whole homogenate of prostate was measured.

propionate into young mature rats castrated 7 days induced a rapid increase in ODC activity. ODC activity was restored to about 40% and 90% relative to untreated young maturte rats after 1 day and 7 days of testosterone-treatment, respectively (Fig. 3).

In contrast, the response of ventral prostate ODC activity of old rats (100W) to castration and testosterone-treatment after castration was significantly slower than that of young mature rats. ODC activity of old rats castrated for 48 hr was diminished about 30% relative to that of untreated old rats(Fig. 2). ODC activity became undetectable 7 days after castration. ODC activity of old rats castrated 7 days remained undetectable after 1 day of testosterone-treatment, and was restored to about 40% relative to untreated rats after 7 days of treatment (Fig. 3).

When tissue DHT content was measured and compared with ODC activity of the same tissue, apparent mutual relation was observed in the ventral prostate of young mature rats (Fig. 4). By contrast, although tissue DHT content of old rats retained a similar level to young rats, ODC activity of old rats did not correlated with tissue DHT content.

DISCUSSION

In this report we document age-related decrease in ODC activity of rat ventral prostate. The result is consistent with the data reported by Shain and Moss using AXC rats[10]. They also showed that the age-related decrease in

ODC activity could not be attributed to change in the properties of enzyme by aging or to age-related production of inactivators or inhibitors of the enzyme[10].

Prostatic ODC activity of both young and old rats responded to androgen withdrawal and to stimulation by androgen after castration. However, the time course of the responses were significantly slower in old rats than in young mature rats. These results suggest that androgen-dependency of prostatic ODC activity is affected by aging.

It could be argued that the age-related alteration in ODC activity of rat ventral prostate was resulted from changes in the amount of testosterone which could be converted to DHT by 5α-reductase in prostatic cells. However, our results showed that prostatic DHT content of old rats retained a similar level to that of young mature rats. Moreover, Shain and Nitchuk have shown that ventral prostate of old rats retain at least 60% of the 5α-reductase activity of young mature rats and the diminished activity is reversed by exogenous testosterone[9]. Boesal et al have shown that testosterone treatment restores the diminished androgen receptor content of prostates of old rats to levels indistinguishable from those in prostates of young mature rats[2]. These findings together with the fact, testosterone administration to castrated rats for up to 7 days cannot induce the ODC activity of old rats to that of young mature rats, suggest that age-related alteration of androgen dependency of ODC activity in ventral prostate may not be the consequence of decreased production of DHT in prostatic cells but disorder in the process following androgen-receptor complex formation or change in gene structure.

REFERENCE

1. **Beavan, M. A., Wilcox, G. and Terpstra, G. K.** 1978. A microprocedure for the measurement of $^{14}CO_2$ release from ^{14}C-carboxy-labeled amino acids. Anal. Biochem. **84**: 638—641.
2. **Boesel, R. W., Klipper, R. W. and Shain, S.A.** 1980. Androgen regulation of androgen receptor content and distribution in the ventral and drsolateral prostate of aging AXC rats. Steroids **35**: 157—177.
3. **Cohen, S. S.** 1971. Introduction to the polyamine. Pretice-Hall, New Jersey.
4. **Fuller, D. J. M., Donaldson, L. J. and Thomas, G. H.** 1975. Ornithine decarboxylase activity and ^{125}I-iododeoxyuridine in rat prostate. Biochem. J. **150**: 557—559.
5. **Janne, J., Poso. H. and Raina A.** 1978. Polyamines in rapid growth and cancer. Biochem. Biophys. Acta. **473**: 241—293.
6. **Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265—275.
7. **Pegg, A. E. and Williams-Ashman, H. G.** 1968. Rapid effects of testosterone on prostatic polyamine-synthesizing enzyme systems. Biochem. J. **109**: 32—33.
8. **Pegg, A. E., Lockwood, D. H. and Williams-Ashman, H. G.** 1970. Concentrations of putrescine and polyamines and their enzymic synthesis during androgen-induced prostatic growth. Biochem. J. **117**: 17—31.
9. **Shain, S. A. and Nitchuk, W.M.** 1979. Testosterone metabolism by the prostate of the aging AXC rat. Mech. Aging. Dev. **11**: 9—22.
10. **Shain, S. A. and Moss, A. L.** 1981. Aging in the AXC rat: Differential effects of testosterone treatment on restoration of diminished prostate L-ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities. Endocrinology **109**: 1184—1191.