Effect of Aging, Castration, and Testosterone Administration on Ribonuclease and Ribonuclease-Inhibitor Activities in the Prostate of Rats

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Free ribonuclease (RNase)-inhibitor activities in both ventral and dorsal prostates had their highest peaks in 4-week-old rats and smallest peaks in around 7-week-old animals. Total RNase activity in the ventral prostate decreased overall with age, while that in the dorsal prostate increased. No significant amount of free RNase activity was found in either prostate.

Weight, protein content, and free RNase-inhibitor activity in both prostates decreased after castration and increased after administration of testosterone to castrated rats. Total RNase activity in the ventral prostate was increased by castration and decreased by testosterone administration. In the dorsal prostate, total RNase activity had two peaks, 7 d after castration and 2 d after testosterone administration. A large amount of free RNase activity was found in the ventral prostate 7 d after castration and this activity was decreased by testosterone administration. In the dorsal prostate, free RNase activity was not detected after castration and testosterone administration.

These results suggest that changes in the level of RNase-inhibitor in both prostates are involved in the regulation of their RNA content through the control of free RNase activity.

Keywords RNase-inhibitor; RNase; prostate; aging; castration; testosterone; rat

Introduction

The presence of ribonuclease (RNase) inhibitors has been demonstrated in many mammalian tissues.^{1,2)} These inhibitors are acidic proteins with a molecular weight of about 5×10^4 .²⁾ They inhibit bovine pancreatic RNase A noncompetitively by forming 1:1 complexes.³⁾ The inhibitors are inactivated by SH reagents, *e.g.*, *p*-chloromercuribenzoic acid and *p*-chloromercuribenzenesulfonic acid. The treatment of RNase-inhibitor complexes with SH reagents regenerates active RNases from the complexes.¹⁾

The precise physiological role of RNase inhibitors remains unclear. However, elevated levels of the inhibitor activity were seen in rapidly growing cells such as regenerating rat liver⁴⁾ and phytohemagglutinin-stimulated cultured human lymphocytes.⁵⁾ Therefore, RNase inhibitors have been suspected to be involved in the regulation of ribonucleic acid (RNA) and protein syntheses.^{6–8)} In addition, an increase in RNase-inhibitor activity was found in the rat adrenal gland stimulated by adrenocorticotrophic hormone.⁹⁾ Furthermore, the inhibitor activities in rat thyroid and uterine were increased by the administration of thyroid-stimulating hormone¹⁰⁾ and estrogen,¹¹⁾ respectively. The activity of RNase was decreased by hepatectomy,⁴⁾ phytohemagglutinin stimulation,⁵⁾ and administration of various hormones.^{9–11)} RNase-inhibitor in the uterus of immature rats was present in a greater amount than RNase, but free inhibitor was no longer detectable in the uterine cytoplasm after precocious treatment with estrogen^{12,13)} or during the course of normal development.¹⁴⁾ After administration of estrogen to ovariectomized rats, RNase-inhibitor activity disappeared from uterine cytosol.¹⁵⁾ These results appear to be contradictory to the above proposed role of the RNase-inhibitor, because the elevated RNase activity results in a decrease in RNA and protein syntheses. With respect to RNase-inhibitor in male gonads, RNase-inhibitor has been purified from rat testis and characterized,¹⁶⁾ but its relation to maturation or sex hormone treatment has not been examined. The relationship between androgen administration and tissue RNase activity has been studied only in mouse skeletal muscle and no significant effect of androgen

was demonstrated.¹⁷⁾

We found an RNase-inhibitor in rat prostate and isolated it as a protein about 4×10^4 in molecular weight (unpublished data). In the present paper, we describe the effect of aging, castration, and androgen administration following castration on this RNase-inhibitor and RNase activities in rat ventral and dorsal prostates.

Materials and Methods

Materials Testosterone propionate and *p*-chloromercuribenzenesulfonic acid were purchased from Nakarai Tesque Inc. Sesame oil was obtained from Kanto Chemical Co., Inc. Bovine pancreatic RNase A (type II-A) and yeast RNA (type VI) obtained from Sigma Chemical Co. were purified according to the methods of Hirose *et al.*¹⁸⁾ and Kumagai *et al.*,¹⁹⁾ respectively. Laboratory chow (MF) was purchased from Oriental Yeast Co., Ltd.

Animals and Their Treatments Male Wistar rats (2–7 weeks old) were purchased from Takasugi Experimental Animal Co., Ltd. They were maintained in a temperature ($22 \pm 2^\circ\text{C}$)-controlled room with 12 h light–12 h dark cycle (light on from 7.00 a.m.), and received laboratory chow and tap water *ad libitum*. The rats under the above conditions were kept for 4–10 d after purchase, and healthy rats with normal body weight gains were used in the experiment. Castration was performed by the scrotal route under ether anesthesia using 7-week-old rats. Testosterone propionate dissolved in sesame oil (20 mg/ml) was injected subcutaneously to castrated rats at 9.30 a.m.

Preparation of 100000 $\times$ g Supernatant (S-100) from Rat Prostate Preparation of S-100 was performed essentially as described previously.²⁰⁾ Rats were exsanguinated without anesthesia. After the livers were perfused with isotonic saline, the prostates were removed quickly and rinsed in an ice-cold solution containing 20 mM Tris–HCl (pH 7.5), 200 mM sucrose, 100 mM NH_4Cl , 5 mM magnesium acetate, and 60 mM 2-mercaptoethanol. All subsequent steps were carried out at $0\text{--}4^\circ\text{C}$. The rinsed prostates were homogenized in 2.5 vols. of the above solution using a glass homogenizer. The homogenate was centrifuged for 20 min at $17000 \times g$. The resulting supernatant fraction was centrifuged for 90 min at $105000 \times g$ to obtain S-100. The S-100, after being dialyzed against two changes of 1.5 l of a solution containing 20 mM Tris–HCl (pH 7.5), 20% glycerol, and 2 mM dithiothreitol, was used for the enzyme assay.

RNase and RNase-Inhibitor Assays Assay of free RNase activity was performed according to the procedure described previously.²¹⁾ The reaction mixture (0.2 ml) contained 50 mM Tris–HCl (pH 7.5), 20 mM ethylenediaminetetraacetic acid (EDTA), 200 μg of yeast RNA, and S-100. After incubation of the reaction mixture at 37°C for 60 min, the reaction was terminated by the addition of 0.2 ml of 5% perchloric acid containing 0.25% uranyl acetate. The mixture was centrifuged after being cooled in

an ice-bath for 20 min, and the resulting supernatant fraction was diluted with 4 vols. of distilled water. The acid-soluble nucleotides were measured at 260 nm.

In order to measure the total RNase activity, 40 μ l of 2.5 mM *p*-chloro-mercuribenzenesulfonic acid, which completely inactivates the RNase-inhibitor in S-100, was added to the reaction mixture for the free RNase assay. Other conditions were the same as those described above.

One unit of the free and total RNase activities was defined as the amount of enzyme which caused an increase in absorbance at 260 nm of 0.1 under the above conditions.

Assay of free RNase-inhibitor activity was performed by the method of Shortman²²⁾ with a slight modification. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 20 mM EDTA, 200 μ g of RNA, 20 μ g of bovine serum albumin, 2 ng of RNase A, and S-100. Other conditions were the same as those of free RNase assay except that the incubation was carried out for 30 min.

One unit of free RNase-inhibitor activity was defined as the amount of inhibitor which produced 50% inhibition of 2 ng of RNase A activity under the above conditions.

Protein Estimation Protein determination was performed according to the method of Lowry *et al.*²³⁾ using bovine serum albumin as standard.

Results and Discussion

Changes in Weight, Free RNase-Inhibitor Activity, and Free and Total RNase Activities of Ventral and Dorsal Prostates of Rats at Different Stages of Growth Changes in the prostate weight at different stages of growth are shown in Fig. 1. The weight of ventral and dorsal prostates increased progressively in from 3- to 8-week-old rats.

As shown in Fig. 2, free RNase-inhibitor activities in both prostates had their highest peaks in 4-week-old animals and smallest peaks at around 7-weeks. Free RNase-inhibitor activity in the ventral prostate was higher than that in the dorsal prostate. Matsuo *et al.*²⁴⁾ reported that serum concentration of testosterone in rats began to increase at about 4 weeks after birth. Our results show that ventral and dorsal prostates contain the highest free RNase-inhibitor activity at 4-weeks. Therefore, it is suggested that the increase in free RNase-inhibitor activity in both prostates of 4-week-old rats may be due to the secretion of androgen.

As shown in Fig. 3, total RNase activity in the ventral prostate decreased overall with age, while that in the dorsal prostate increased. Neither the ventral nor the dorsal prostate contained any significant amount of free RNase

activity. Thus, all RNases existed as complex with the RNase-inhibitor in both prostates.

When RNase-RNase inhibitor complex obtained by DEAE-Toyopearl chromatography of S-100 from ventral and dorsal prostates was analyzed on sodium dodecyl-sulfate polyacrylamide gels by activity staining, two bands of RNase activity with molecular weights of 14000 and 16000 were detected in the dorsal prostate. In the ventral prostate, however, only one band with molecular weight of 14000 was observed. One possible explanation for the difference in changes in total RNase activity with age between the two prostates is as follows. In the ventral prostate, the decrease in activity might be due to the decrease in RNase with a molecular weight of 14000, while in the dorsal prostate, the increase in activity might be due to the increase in RNase with a molecular weight of 16000 rather than the change in RNase with a molecular weight of 14000.

Effect of Castration and Testosterone Administration on Weight, Protein Content, Free RNase-Inhibitor Activity, and Free and Total RNase Activities of Ventral and Dorsal

Fig. 1. Changes in Rat Prostate Weight at Different Stages of Growth

Rats used were 41.2 g (3-week-old), 81.2 g (4-week-old), 119.1 g (5-week-old), 181.2 g (6-week-old), 258.4 g (7-week-old), and 306.0 g (8-week-old) mean body-weight. Ventral (●) and dorsal (○) prostates from 1–14 rats were pooled separately for each experimental group. Each point represents mean $\pm$ S.E.M. of 3–5 experiments.

Fig. 2. Changes in Free RNase-Inhibitor Activity in Rat Prostates at Different Stages of Growth

Rats used were the same as described in the legend to Fig. 1. Ventral and dorsal prostates from 1–14 rats were pooled separately for each experimental group. S-100 was prepared from each prostate sample and assayed for free RNase-inhibitor activity as described in Materials and Methods. Each point represents mean $\pm$ S.E.M. of 3–5 experiments.

Fig. 3. Changes in Free and Total RNase Activities in Ventral (A) and Dorsal (B) Prostates from Rats at Different Stages of Growth

Rats used were the same as described in the legend to Fig. 1. S-100 was prepared from each prostate sample and assayed for free and total RNase activities as described in Materials and Methods. Each point represents mean $\pm$ S.E.M. of 3–5 experiments.

Fig. 4. Effect of Castration and Testosterone Administration on Weight and Protein Content of Rat Prostates

Seven-week-old rats (258.4 g mean body-weight) were castrated. At 1, 2, 4, and 7 d after castration, they were sacrificed by exsanguination and ventral and dorsal prostates were excised from 1–5 animals for each experimental group. Each prostate sample was weighed and protein content determined as described in Materials and Methods.

Starting on the seventh day after castration, rats were injected subcutaneously with 2 mg of testosterone propionate per 100 g body weight every 24 h. They were sacrificed at 1, 2, 4, 7, 10, and 14 d after the start of testosterone treatment, and ventral and dorsal prostates were excised from 1–5 animals for each experimental group. Weight of ventral and dorsal prostates and their protein content were measured. Each point represents mean $\pm$ S.E.M. of 3–5 experiments.

Prostates of Rats As shown in Fig. 4, a significant decrease in weight and protein content of the two prostates was observed following castration, but the weight was increased by testosterone administration. Weight of the ventral prostate returned to control value (the value just prior to castration) between 7 and 8 d after the beginning of testosterone administration, while that of the dorsal prostate returned to control value after just 2–3 d. Protein content of both prostates increased 1 or 2 d after testosterone administration and no remarkable changes were observed thereafter. As shown in Fig. 5, free RNase-inhibitor activity in both glands decreased after castration and by 7 d had disappeared from the ventral prostate. No free RNase-inhibitor activity was detected in this prostate

Fig. 5. Effect of Castration and Testosterone Administration on Free RNase-Inhibitor activity of Rat Prostates

Rats used and the treatment of animals were the same as described in the legend to Fig. 4. S-100 was prepared from each prostate sample and assayed for free RNase-inhibitor activity as described in Materials and Methods. Each point represents mean $\pm$ S.E.M. of 3–5 experiments.

1 d after the beginning of testosterone administration, but activity was restored almost to control value after 10 d of daily testosterone injection. In the dorsal prostate, free RNase-inhibitor activity was not detected 2 d after the beginning of testosterone administration, but after 7 d, maximal activity was observed; this was followed by a marked fall within a few days.

As shown in Fig. 6, total RNase activity in the ventral prostate was increased by castration and decreased by testosterone administration. Free RNase activity in this prostate was not detected until 4 d after castration, but after 7 d, a large amount of the activity was found. The value of this activity was almost equal to that of total RNase activity, suggesting that RNase did not exist as complex with RNase-inhibitor 7 d after castration. The free RNase activity in the ventral prostate of castrated rats was decreased by testosterone administration. In the dorsal

Fig. 6. Effect of Castration and Testosterone Administration on Free and Total RNase Activities of Rat Prostates

Rats used and the treatment of animals were the same as described in the legend to Fig. 4. S-100 was prepared from each prostate sample and assayed for free and total RNase activities as described in Materials and Methods. Each point represents mean $\pm$ S.E.M. of 3–5 experiments.

prostate, total RNase activity had two peaks: one was observed 7 d after castration and the other 2 d after the beginning of testosterone administration. Free RNase activity was not found in the dorsal prostate after castration and the subsequent treatment.

Our results suggest that changes in the levels of free RNase-inhibitor activity and free and total RNase activities in the ventral prostate are dependent on the amount of androgen, and that this is also true in the dorsal prostate but to a lesser degree.

Coffey *et al.*²⁵⁾ reported that RNA content in the ventral prostate decreased after castration and increased after injection of testosterone to castrated rats. In the present study, castration decreased the free RNase-inhibitor activity in the animals but increased free RNase activity in this prostate. However, with the growth response to androgenic stimulation of the ventral prostate, free RNase-inhibitor activity increased and free RNase activity decreased. Our results therefore suggest that changes in the level of RNase-inhibitor in ventral and dorsal prostates are involved in the regulation of their RNA content through the control of free RNase activity.

It was reported that after injection of labeled testosterone to castrated rats, uptake of the radioactivity by the ventral prostate was 3 times higher than that by the dorsal prostate.²⁶⁾ Our data show that changes in the levels of weight, protein content, free RNase-inhibitor activity, and free and total RNase activities in the dorsal prostate were all smaller than those in the ventral prostate following testosterone administration. This difference in the two glands might be due to the difference in the uptake of testosterone.

Shimazaki *et al.*²⁷⁾ and Moore and Wilson²⁸⁾ reported that 5α -reductase activity in the rat ventral prostate was decreased by castration and increased by testosterone administration. Mainwaring and Mangan²⁹⁾ reported that the amount of dihydrotestosterone receptor protein decreased in the rat ventral prostate after castration. These results suggest that changes in the levels of 5α -reductase

activity and amount of dihydrotestosterone receptor are dependent on the amount of testosterone in the rat ventral prostate. Therefore, there is another possibility that the difference in weight, protein content, free RNase-inhibitor activity, and free and total RNase activities between ventral and dorsal prostates might be due to differences in the activity of 5α -reductase and the amount of dihydrotestosterone receptor in the two glands.

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