Active Principle of Swine Prostate Extract: II. Effect of a Peptide Isolated from Swine Prostate Extract on Rat Prostate

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Received October 31, 1990 Accepted May 1, 1991

ABSTRACT—Previously, we purified a substance from swine prostate extract (PE) that had been reported to have therapeutic effect on benign prostatic hypertrophy. The purified substance (PPE) suppressed $^3$H-testosterone uptake into the prostate in castrated rats. The present study was carried out to examine the effect of PPE on the weight of accessory sexual organs including the prostate and biochemical parameters in the prostate of normal and/or castrated and testosterone-treated rats. 1) In normal rats, the p.o. administration of PPE daily for a total of 30 days did not affect the prostate weight, but reduced the citric acid content in the prostate. The treatment had little or no influence tissue $O_2$ uptake, aconitase activity or isocitrate dehydrogenase activity in the prostate. 2) In castrated and testosterone-treated rats, the p.o. treatment with PPE for 15 or 30 days reduced the weight of the prostate as well as the total citric acid, DNA and RNA contents in prostatic tissue. However, these biochemical parameters per tissue weight were not obviously affected except for the citric acid content. These findings suggest that PPE is one of the active principals of PE for the therapeutic efficiency on benign prostatic hypertrophy, probably due to its suppressive effect on excessive uptake of androgen by the prostate.

It has been reported (1–4) that extracts from animal prostates have a therapeutic effect on benign prostatic hypertrophy. Previously, we reported that the cattle and/or swine prostate extracts had the activity to enhance human prostatic acid phosphatase (PAPase) activity in vitro (5) and could increase the muscular tonicity of the urinary bladder by directly acting upon vesical muscles, suggesting that the extracts have an activity to elevate the intravesical voiding pressure in vivo (6). In the preceding paper (7), we isolated and purified an active principle, which activated PAPase, from swine prostates (PE). The purified PE (PPE) was a peptide with a molecular weight of about 8,800 that was composed largely of neutral amino acids (approximately 70%) and a few aromatic amino acids. PPE activated PAPase in a dose-dependent fashion as well as recovered PAPase activity from the inhibition by L-tartaric acid. Furthermore, the oral administration of PPE suppressed uptake of $^3$H-testosterone into the prostate of castrated rats (7). These results suggested that PPE was one of the active principles of PE for the therapeutic effect on prostatic hypertrophy.

Several groups of investigators have discussed from the metabolic viewpoint about the
pathogenesis of benign prostatic hypertrophy. Barron and Huggins (8) have described that hypertrophic prostatic tissue shows depressed tissue respiration, low metabolic utilization of pyruvate, abnormal carbohydrate metabolism and enhanced anaerobic glycolysis. A high content of citric acid has been also demonstrated in hypertrophic prostate. Kanazawa et al. (9) reported that the increased citric acid content might be ascribed to low aconitase activity in the TCA cycle, while Samuels and Harding (10) and Cooper and Imfeld (11) inferred that it was primarily due to decreased isocitrate dehydrogenase activity. Although there is still much debate about the cause of prostatic hypertrophy, it is generally accepted that the pathogenesis of prostatic hypertrophy occurs through an undue accumulation of testosterone or dihydrotestosterone in the prostate. In fact, Shida et al. (12) have reported that gestagenic compounds show a gratifying therapeutic effect on human benign prostatic hypertrophy.

It is known that atrophy of prostates in castrated rats is recovered by the administration of testosterone (13). In the present paper, therefore, studies were carried out to examine the effects of PPE on the weights of accessory sexual organs and biochemical parameters of the prostate in normal rats as well as the testosterone-induced recovery of prostates in castrated rats.

MATERIALS AND METHODS

Materials

PPE was purified from swine prostate extract (PE) as described previously (7). The following materials were also employed: testosterone propionate (testosterone), trichloroacetic acid (TCA), sodium citrate, sucrose, sodium isocitrate, acetic anhydride and pyridine (Nacalai Tesque, Kyoto, Japan); ribonucleic acid type XI (RNA, Kohjin, Tokyo, Japan); and nicotinamide dinucleotide (NADP) and deoxyribonucleic acid type VIII (DNA) (Sigma, St. Louis, MO).

Animals

Male Wistar rats, 18 weeks old, were obtained from Kitayama Labesu (Ina, Nagano, Japan). The animals were used for the experiments after an acclimatization for more than 7 days. They were housed in an air-conditioned animal room at 20°C with 55 ± 5% relative humidity, and they were given water and pelleted food ad libitum.

Treatment of rats with PPE or PE

PPE and PE were dissolved in water at an appropriate concentration for oral administration with a volume of 0.2 ml/10 g body weight. Rats were given PPE or PE intragastrically via a gastric tube. Control animals were received water as a vehicle.

Castration and testosterone-treatment of rats

Rats were subjected to bilateral orchectomy under anesthesia with ether. The castrated rats were given testosterone dissolved in olive oil in a dose of 0.15 mg/kg/day, s.c. in the back every day starting 8 days after the operation to the day before the killing of the rats.

Isolation of accessory sexual organs

The rats examined were killed by bleeding under anesthesia with ether, and the prostate and seminal vesicle were isolated. The prostate was separated into the dorsal and ventral lobes. Muscle levator ani was also isolated if necessary.

Assays of biochemical parameters in dorsal and ventral lobes of the prostate

Results were expressed as the amount (μg or unit) per 100 mg tissue weight and/or that per 100 g body weight unless otherwise indicated. Number of samples in each assay is indicated in the legend of each Table or Figure, because the size of the prostate obtained from an individual animal was not enough for all assays.

Citric acid content (14): A 10% homogenate of the prostate was prepared in 5% TCA solution and centrifuged at 4,610 × g for 15 min at
2°C. To 1.0 ml of the resulting supernatant, 8.0 ml of acetic anhydride was added. The mixture in a tightly stoppered container was heated at 60°C for 10 min. Then, 1.0 ml of pyridine was added; and after thoroughly mixing, the mixture was reheated at 60°C for 40 min. After cooling, the absorbance at 420 nm was determined. The citric acid content was estimated by reference to a calibration curve using standard citric acid solutions.

O₂ uptake (QO₂): After rinsing with Krebs-Ringer phosphate buffer (pH 7.4), the prostate was minced to a size of 0.4 mm with a tissue chopper, and the oxygen uptake of 100 mg of the minced tissue was measured in a Warburg manometer, using 5 × 10⁻³ M glucose as the substrate. The substrate solution (3 ml) was applied to the apparatus and 0.2 ml of 20% KOH solution was placed in the accessory chamber. The measurement was carried out six times at 20-min intervals for 2 hours at 37°C with 80 shakes/min. QO₂ was expressed as the volume of O₂ (μl)/100 mg tissue weight/hr.

Aconitase activity (15): The prostate specimen was homogenized in five volumes of 0.25% sucrose solution containing 10⁻³ M sodium citrate. The homogenate was centrifuged at 31,000 × g for 30 min at 2°C, and the resulting supernatant (assay sample) was measured for the aconitase activity. The enzymatic reaction mixture containing 0.05 ml of the assay sample, 0.3 ml of 0.2 M sodium citrate, 1.15 ml of water and 2.0 ml of 0.1 M tris-HCl buffer (pH 7.4) was incubated at 25°C. The absorbance of the mixture at 240 nm was measured 5 times at an interval of 1 min against the blank, in which the substrate solution was replaced with water. One unit of the enzyme activity is defined as an increase in absorbance by 0.001 in a minute.

Isocitrate dehydrogenase activity (16): A 10% homogenate of the prostate was prepared in 0.01 M phosphate buffer (pH 7.4) and centrifuged at 39,800 × g for 30 min at 2°C. Isocitrate dehydrogenase activity of the supernatant was assayed by the enzymatic reaction. The reaction mixture containing 0.1 ml of the assay sample, 0.2 ml of 1.5 × 10⁻³ M NADP, 0.2 ml of 2 × 10⁻² M MnCl₂, 0.2 ml of 2 × 10⁻² M sodium citrate, 1.3 ml of water and 1.0 ml of tris-HCl buffer (pH 7.4) was incubated at 25°C. The absorbance at 340 nm of the mixture was measured 6 times at a 1-min interval against the blank, in which the substrate solution was replaced with water. One unit of the enzyme activity is defined as an increase in absorbance by 0.001 in a minute.

DNA and RNA contents: DNA and RNA contents were assayed according to the method of Schneider (17). Briefly, a 10% homogenate of prostate (100 mg) was prepared in ice-cold 5% TCA solution and washed by centrifugation (31,000 × g, 30 min, 2°C) with 4 ml of ice-cold 10% TCA solution to remove the acid soluble fraction. The precipitate was washed twice by centrifugation with 76 and 95% ethyl alcohol to remove TCA. The precipitate was boiled 3 times for 3 min with each 5 ml mixture of ethylalcohol and ether (3:1). After centrifugation, the precipitate was suspended in 3 ml of 5% TCA and heated at 90°C. The absorbance of the reaction mixture was determined at 600 nm. For the DNA assay, one ml of the TCA-soluble fraction obtained was mixed with 2 ml of diphenylamine reagent and heated in boiling water for 20 min. The absorbance of the reaction mixture was determined at 600 nm. For the RNA assay, 1.5 ml of the TCA soluble fraction was mixed with 1.5 ml of orcinol reagent and heated in boiling water. The absorbance of the reaction mixture was determined at 660 nm. The concentrations of DNA and RNA in the solution were calculated by reference to the respective calibration curves using the standard DNA and RNA.

Statistical analysis

The results were expressed as the mean ± S.E. Data were statistically evaluated using Student's t-test. A difference at the level of P < 0.05 was considered to be significant.
RESULTS

Effect of PPE on the accessory sexual organs of normal rat

Rats (12 or 13 animals in each group) received PPE, p.o. for 30 days in doses of 0.01 to 1.0 mg/kg/day. The tissues obtained on the day after the final treatment were subjected to the following assays.

Body weight and accessory sexual organ weights: PPE hardly affected the weights of the dorsal and ventral lobes of the prostate, the weight of the seminal vesicle, and the body weight (Table 1).

Citric acid content in prostate: The dorsal lobe of the control exhibited higher citric acid content than the ventral lobe (Fig. 1). PPE reduced the citric acid content of the dorsal lobe almost dose-dependently in both levels per 100 mg tissue weight and per 100 g body weight (Fig. 1, left panel). The citric acid content of the ventral lobe per 100 g body weight was also reduced by PPE, although the reduction was not dose-dependent (Fig. 1, right panel).

O₂ uptake, aconitase activity and isocitrate dehydrogenase activity in the prostate: As shown in Table 2, the QO₂ of the dorsal lobe in the control was less than that of the ventral lobe. There was little or no difference of QO₂ value between the control and any of the

### Table 1. Effect of purified prostate extract (PPE) on body weight and accessory sexual organ weight of normal rats

| (mg/kg) | Body weight (g) | Prostate | Seminal vesicle |
|---------|----------------|----------|-----------------|
|         |                | dorsal lobe | ventral lobe | dorsal lobe | ventral lobe |
| Control | 442 ± 17       | 72.0 ± 2.4  | 114.8 ± 9.5  | 120.2 ± 5.9 |
| PPE     | 0.01 444 ± 15  | 77.3 ± 5.6  | 114.9 ± 5.4  | 124.3 ± 6.7 |
|         | 0.03 450 ± 19  | 70.4 ± 4.9  | 106.4 ± 8.5  | 102.2 ± 6.7 |
|         | 0.1 444 ± 16   | 70.7 ± 5.4  | 111.5 ± 7.0  | 107.4 ± 6.1 |
|         | 0.3 467 ± 16   | 75.7 ± 5.4  | 98.3 ± 4.9   | 112.4 ± 6.0 |
|         | 1.0 450 ± 15   | 77.2 ± 4.5  | 111.0 ± 5.1  | 118.2 ± 5.7 |

PPE was given p.o. for 30 days (N = 12 or 13 animals).

### Table 2. Effect of purified prostate extract (PPE) on O₂ uptake, aconitase activity and isocitrate dehydrogenase activity in the prostate of normal rats

| (mg/kg) | O₂ uptakeᵃ | Aconitaseᵇ | Isocitrate dehydrogenaseᶜ |
|---------|------------|------------|---------------------------|
|         | dorsal lobe | ventral lobe | dorsal lobe | ventral lobe | dorsal lobe | ventral lobe |
| Control | 13.0 ± 1.0  | 30.5 ± 5.0  | 100 ± 5      | 101 ± 5      | 663 ± 34    | 429 ± 36    |
| PPE     | 0.01 17.5 ± 2.8 | 33.2 ± 6.7 | 92 ± 5      | 98 ± 8      | 683 ± 25    | 411 ± 29    |
|         | 0.03 14.3 ± 2.3 | 24.9 ± 3.7 | 88 ± 4      | 88 ± 6      | 694 ± 19    | 424 ± 29    |
|         | 0.1 12.7 ± 2.8 | 26.9 ± 3.6 | 81 ± 6      | 83 ± 10     | 694 ± 25    | 397 ± 28    |
|         | 0.3 15.6 ± 1.4 | 33.0 ± 2.7 | 90 ± 6      | 105 ± 6     | 693 ± 22    | 442 ± 25    |
|         | 1.0 15.8 ± 1.3 | 28.7 ± 4.7 | 86 ± 5      | 85 ± 3*     | 722 ± 24    | 462 ± 33    |

ᵃ): μl O₂/100 mg tissue weight/hr (N = 5 animals). b): unit/100 mg tissue weight (N = 8 animals). c): unit/100 mg tissue weight (N = 7 animals). PPE was given p.o. for 30 days (N = 5 animals). *: Statistically significant difference from the control at P < 0.05.
PPE-treated groups both in the dorsal and ventral lobes. The aconitase activity in the dorsal lobe of the control was almost the same level as that of the ventral lobe. The isocitrate dehydrogenase activity of the dorsal lobe of the control was higher than that of the ventral lobe. There was no appreciable difference of these enzyme activities between the control and any of the PPE-treated groups.

Fig. 1. Effect of purified prostate extract (PPE) on citric acid content in the prostate of normal rats. Drugs were given p.o. for 30 days. Each column represents the mean ± S.E. of 4 or 5 animals. *: Statistically significant difference from the control at P < 0.05.

Table 3. Recovery of accessory sexual organ weights by testosterone in castrated rats

| Dose of testosterone (mg/kg) | mg tissue weight/100 g body weight | Prostate | Seminal vesicle |
|------------------------------|-----------------------------------|----------|----------------|
|                              | dorsal lobe | ventral lobe |                     |
| Normal rats                  |            |                |                     |
| Castrated rats               |            |                |                     |
| 0.1                          | 82.7 ± 2.4 | 94.5 ± 13.0    | 104.6 ± 10.2        |
| 0.2                          | 65.1 ± 4.2 | 77.1 ± 7.1     | 82.2 ± 8.3          |
| 0.5                          | 83.4 ± 4.3 | 112.7 ± 17.2   | 125.6 ± 14.2        |
| 125.4 ± 5.1                  | 151.0 ± 20.9 | 172.8 ± 3.8    |                     |

Castrated rats were given testosterone every day in doses of 0.1 to 0.5 mg/kg for 30 days starting 8 days after the castration. Each group included 3 animals. *: Statistically significant difference from normal rats at P < 0.05 and P < 0.01, respectively.

Effect of PPE and PE on the accessory sexual organs of castrated and testosterone-treated rats

As a preliminary experiment, castrated rats were treated with 0.1 to 0.5 mg/kg/day of testosterone, s.c., every day for 30 days, starting 8 days after the operation. From the results of the experiment (Table 3), 0.15 mg/kg/day of testosterone was used in the following experiment to restore their accessory sexual organ...
weights to the normal level.

To examine the effect of PPE on the accessory sexual organs of castrated and testosterone-treated rats, the castrated animals were given PPE (0.1 to 3 mg/kg) or PE (100 mg/kg), p.o. daily in combination with testosterone starting 8 days after the operation to the day before the killing of the rats. The animals were killed after 15-day or 30-day treatments. Some of the castrated rats and normal rats were killed 8 days after the operation.

**Body weight and accessory sexual organ weights:** The body weight of castrated and testosterone-treated rats as the control increased significantly by days 15 and/or 30 of the treatment with testosterone (Table 4). PPE and PE did not affect the body weight gain.

As shown in Table 5 (dorsal and ventral lobes of prostate) and Table 6 (seminal vesicle and M. levator ani), the weights of accessory sexual organs decreased significantly 8 days after the castration.

The treatment of the castrated rats with testosterone recovered the weights of the dorsal and ventral lobes as well as that of the seminal vesicle by the 15-day and 30-day treatments. The 15-day treatment with testosterone did not recover the M. levator ani weight, but the 30-day treatment recovered it.

As shown in Table 5, the 15-day combined treatments with 1.0 and 3.0 mg/kg of PPE and with 100 mg/kg of PE decreased the dorsal lobe weight significantly (82, 84 and 83% of the control). Also, the 30-day treatments with 0.3 to 3.0 mg/kg of PPE and 100 mg/kg of PE suppressed it significantly (77–82% of the control). The 15-day and 30-day treatment with higher doses of PPE showed a tendency to suppress the ventral lobe weight.

As shown in Table 6, the 15-day and 30-day treatments with PPE in higher doses and with 100 mg/kg of PE decreased or showed a tendency to decrease the seminal vesicle weight. The 15-day combined treatment with either PPE or PE did not decrease the weight of the M. levator ani, but the 30-day treatment with PPE in doses of 1.0 and 3.0 mg/kg decreased it significantly (79 and 86% of the control).

**DNA content in prostate:** As shown in Table 7, on day 8 after the castration, the amounts of DNA per 100 mg tissue weight in the dorsal

### Table 4. Effects of purified prostate extract (PPE) and prostate extract (PE) on body weight of castrated and testosterone-treated rats

| Dose (mg/kg) | 15-day treatment (%) | 30-day treatment (%) |
|--------------|----------------------|----------------------|
| Normal²     | 387 ± 8              |                      |
| Castrated²  | 380 ± 13             |                      |
| Control³⩙   | 422 ± 12² (100)      | 465 ± 12² (100)      |
| PPE          | 423 ± 12 (100)       | 474 ± 15 (102)       |
| 0.3          | 425 ± 15 (101)       | 476 ± 11 (102)       |
| 1.0          | 427 ± 11 (101)       | 482 ± 13 (104)       |
| 3.0          | 422 ± 11 (100)       | 475 ± 12 (102)       |
| PE           | 418 ± 11 (99)        | 473 ± 13 (102)       |

*²: Measured 8 days after the castration (N = 9 animals). *³: Treated with testosterone (0.15 mg/kg/day, s.c.) for 15 or 30 days starting 8 days after the castration (N = 10 animals). *⩙: PPE or PE was given p.o. in combination with testosterone for 15 or 30 days (N = 9 or 10 animals). *²⁻²: Statistically significant difference from the castrated rats at P < 0.05 and at P < 0.01, respectively.
Table 5. Effects of purified prostate extract (PPE) and prostate extract (PE) on weights of dorsal and ventral lobes in the prostate of castrated and testosterone-treated rats

| Dose (mg/kg) | 15-day treatment | 30-day treatment | 15-day treatment | 30-day treatment |
|--------------|------------------|------------------|------------------|------------------|
| Normal       |                  |                  |                  |                  |
| Castrated    |                  |                  |                  |                  |
| Control      | 60.6 ± 3.11      | (100)            | 63.8 ± 4.1           | (100)           | 79.1 ± 6.1        | (100)            | 87.3 ± 5.3        | (100)           |
| PPE          | 0.51            | (94)             | 53.7 ± 2.9         | (84)             | 65.8 ± 8.0        | (83)             | 81.9 ± 2.2        | (94)             |
|              | 0.8             | (97)             | 52.5 ± 2.5         | (82) *           | 83.3 ± 5.8        | (105)            | 87.0 ± 6.0        | (100)            |
|              | 1.0             | (82) *           | 49.7 ± 3.4         | (78) *           | 68.7 ± 3.7        | (87)             | 72.9 ± 4.7        | (84)             |
|              | 3.0             | (84) *           | 51.5 ± 2.7         | (81) *           | 63.8 ± 4.1        | (81)             | 72.9 ± 5.8        | (84)             |
| PE           | 100             | (83) *           | 49.2 ± 2.7         | (77)            | 59.7 ± 5.2        | (75)             | 79.9 ± 5.0        | (92)             |

* p < 0.01, #: Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.

Table 6. Effects of purified prostate extract (PPE) and prostate extract (PE) on weights of seminal vesicle and M. levator in the prostate of castrated and testosterone-treated rats

| Dose (mg/kg) | 15-day treatment | 30-day treatment | 15-day treatment | 30-day treatment |
|--------------|------------------|------------------|------------------|------------------|
| Normal       |                  |                  |                  |                  |
| Castrated    |                  |                  |                  |                  |
| Control      | 91.3 ± 6.11      | (100)            | 97.1 ± 5.2        | (100)           | 36.9 ± 4.4        | (100)            | 63.0 ± 2.2        | (100)           |
| PPE          | 0.51            | (93)             | 96.9 ± 4.8        | (100)           | 39.3 ± 5.1        | (107)            | 56.6 ± 3.1        | (90)             |
|              | 0.8             | (95)             | 91.9 ± 4.8        | (95)             | 35.4 ± 4.2        | (96)             | 62.7 ± 2.7        | (100)            |
|              | 1.0             | (86)             | 91.0 ± 7.8        | (94)             | 37.0 ± 3.2        | (100)            | 50.0 ± 3.8        | (79) ^           |
|              | 3.0             | (89) ^           | 84.8 ± 4.9        | (87)             | 39.3 ± 2.9        | (106)            | 54.0 ± 1.1        | (86) ^           |
| PE           | 100             | (92) *           | 90.0 ± 5.6        | (93)             | 41.0 ± 5.0        | (111)            | 56.7 ± 3.6        | (90)             |

* p < 0.01, #: Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.
and ventral lobes of the prostate increased significantly in contrast to the clear decrease in the amounts of DNA per 100 g body weight in these lobes.

The 15-day or 30-day treatments with testosterone reduced the DNA amounts per 100 mg tissue weight in both dorsal and ventral lobes, and they increased those per 100 g body weight significantly.

The 15-day and 30-day combined treatments with PPE did not affect the DNA amount per 100 mg tissue weight in the dorsal and ventral lobes in comparison with the control, but the amount per 100 g body weight in the dorsal lobe was decreased by the 30-day treatment and that in the ventral lobe showed a tendency to be decreased by the 15-day and 30-day treatments. The 30-day PE treatment rather

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### Table 7. Effect of purified prostate extract (PPE) and prostate extract (PE) on the amount of DNA in the prostate of castrated and testosterone-treated rats

|                | Dorsal lobe |                             | Ventral lobe |                             |
|----------------|-------------|-------------------------------|--------------|-------------------------------|
|                | µg/100 mg tissue weight | µg/100 g body weight | µg/100 mg tissue weight | µg/100 g body weight |
|                | 15-day treatment (%) | 30-day treatment (%) | 15-day treatment (%) | 30-day treatment (%) |
| Normal[^a]     | 361 ± 20    | 208 ± 20                      | 376 ± 20     | 417 ± 34                      |
| Castrated[^b]  | 537 ± 17    |                             | 169 ± 15     |                             |
| Control[^c]    | 379 ± 39[^3] | (100) 321 ± 18[^3] (100)     | 238 ± 41     | (100) 219 ± 15[^3] (100)     |
| PPE 0.1[^c]    | 349 ± 31    | (92) 363 ± 53 (113)          | 232 ± 18     | (97) 195 ± 30 (89)           |
| 0.3            | 353 ± 22    | (93) 346 ± 30 (107)          | 240 ± 25     | (101) 180 ± 16 (82)          |
| 1.0            | 383 ± 18    | (101) 307 ± 27 (96)          | 216 ± 22     | (91) 150 ± 6 (69)^[^1]        |
| 3.0            | 386 ± 12    | (101) 328 ± 21 (102)         | 218 ± 13     | (92) 158 ± 25 (72)           |
| PE 100         | 318 ± 17    | (83) 376 ± 12 (117)^[^*]     | 171 ± 8      | (71) 160 ± 9 (73)^[^2]       |

[^a]: Measured 8 days after the castration (N = 5 animals).  
[^b]: Treated with testosterone (0.15 mg/kg/day, s.c.) for 15 or 30 days starting 8 days after the castration (N = 5 animals).  
[^c]: PPE or PE was given p.o. in combination with testosterone for 15 or 30 days (N = 5 animals).  
[^3]: Statistically significant difference from the castrated rats at P < 0.05 and at P < 0.01, respectively.  
[^*]: Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.
increased that per 100 mg tissue weight in the dorsal lobe (117% of the control), but that per 100 g body weight was reduced by the 15-day and 30-day treatments (71 and 73% of the control). The DNA per 100 g body weight in the ventral lobe was reduced by the 15-day treatment.

RNA content in prostate: As shown in Table 8, both the RNA amount per 100 mg tissue weight and that per 100 g body weight decreased significantly in the dorsal and ventral lobes of the prostate on day 8 after the castration, in contrast to the results on DNA.

The testosterone-treatment increased the RNA amount per 100 mg tissue weight and that per 100 g body weight in both the dorsal lobe and ventral lobe.

The RNA amount per 100 mg tissue weight

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Table 8. Effect of purified prostate extract (PPE) and prostate extract (PE) on the amount of RNA in the prostate of castrated and testosterone-treated rats

| Dose (mg/kg) | Dorsal lobe | Ventral lobe |
|--------------|-------------|-------------|
|              | μg/100 mg tissue weight | μg/100 g body weight |
|              | 15-day treatment (%) | 30-day treatment (%) | 15-day treatment (%) | 30-day treatment (%) |
| Normal*1 | 535 ± 25 | P < 0.01 | 310 ± 33 | P < 0.01 |
| Castrated*1 | 398 ± 16 | 128 ± 16 |
| Control*1 | 437 ± 20 (100) | 453 ± 9 (100) | 272 ± 21 (100) | 311 ± 23 (100) |
| 0.1*1 | 441 ± 13 (101) | 469 ± 32 (104) | 296 ± 22 (109) | 252 ± 20 (81) |
| 0.3 | 431 ± 27 (99) | 549 ± 42 (121) | 279 ± 17 (103) | 283 ± 13 (91) |
| 1.0 | 480 ± 27 (110) | 555 ± 114 (100) | 274 ± 35 (101) | 227 ± 15 (73)* |
| 3.0 | 411 ± 19 (94) | 509 ± 22 (112)* | 233 ± 18 (86) | 243 ± 20 (78) |
| PE | 386 ± 13 (88) | 516 ± 25 (113)* | 207 ± 5 (76)* | 220 ± 14 (70)* |

Table 8: Effect of purified prostate extract (PPE) and prostate extract (PE) on the amount of RNA in the prostate of castrated and testosterone-treated rats.

- Normal: Normal rats.
- Castrated: Castrated rats.
- Control: Control rats.
- PPE and PE: Prostate extract.
- 0.1-3.0: Doses of PPE and PE in mg/kg.
- 1070 ± 96: RNA amount in μg/100 mg tissue weight.
- 1185 ± 143: RNA amount in μg/100 g body weight.
- Normal*: RNA amount in μg/100 mg tissue weight for normal rats.
- Castrated*: RNA amount in μg/100 g body weight for castrated rats.

*1: Measured 8 days after the castration (N = 5 animals).
*2: Treated with testosterone (0.15 mg/kg/day, s.c.) for 15 or 30 days starting 8 days after the castration (N = 5 animals).
*3: PPE or PE was given p.o. in combination with testosterone for 15 or 30 days (N = 5 animals).
*4: Statistically significant difference from the castrated rats at P < 0.05 and at P < 0.01, respectively.
*5: Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.
was not appreciably affected by PPE and PE in the 15-day and 30-day treatments. However, the amount per 100 g body weight in the dorsal lobe showed a tendency to be decreased by the 15-day treatment with higher doses of PPE or PE. The amount of RNA per 100 g body weight of the ventral lobe also showed a tendency to be decreased by the 15-day and 30-day treatments with higher doses of PPE.

_Citric acid content in prostate_: As shown in Table 9, the citric acid content per 100 mg tissue weight of the dorsal lobe showed no significant increase on day 8 after the castration (108 ± 11 and 145 ± 14 µg/100 mg tissue weight in normal and castrated rats, respectively), and that of the ventral lobe decreased significantly but slightly (146 ± 10 and 119 ±

| Table 9. Effect of purified prostate extract (PPE) and prostate extract (PE) on citric acid content in the prostate of castrated and testosterone-treated rats |
|-------------------------------------------------|
| **Dorsal lobe** |
| **µg/100 mg tissue weight** | **µg/100 g body weight** |
| **Dose (mg/kg)** | 15-day treatment (%) | 30-day treatment (%) | 15-day treatment (%) | 30-day treatment (%) |
| Normal<sup>a</sup> | 108 ± 11 | 65 ± 7<sup>b</sup> | P < 0.01 |
| Castrated<sup>a</sup> | 145 ± 14 | 41 ± 3<sup>b</sup> |
| Control<sup>c</sup> | 87 ± 12<sup>c</sup> (92) | 116 ± 15<sup>c</sup> (105) | 54 ± 5 (100) | 81 ± 14<sup>c</sup> (100) |
| PPE 0.1<sup>c</sup> | 87 ± 12 (92) | 115 ± 13<sup>c</sup> (99) | 57 ± 7 (106) | 64 ± 11 (83) |
| 0.3 | 82 ± 10<sup>c</sup> (94) | 94 ± 15<sup>c</sup> (81) | 57 ± 11 (106) | 50 ± 10 (83) |
| 1.0 | 77 ± 9<sup>c</sup> (89) | 123 ± 23<sup>c</sup> (106) | 42 ± 2 (78)<sup>c</sup> | 58 ± 8 (79) |
| 3.0 | 84 ± 6<sup>c</sup> (97)<sup>c</sup> | 116 ± 24<sup>c</sup> (101) | 46 ± 3 (85) | 57 ± 12 (77) |
| PE 100 | 69 ± 6<sup>c</sup> (79)<sup>c</sup> | 92 ± 13<sup>c</sup> (79) | 37 ± 3 (69)<sup>c</sup> | 38 ± 45 (67)<sup>c</sup> |

| **Ventral lobe** |
| **Unit/100 mg tissue weight** | **Unit/100 g body weight** |
| **Dose (mg/kg)** | 15-day treatment (%) | 30-day treatment (%) | 15-day treatment (%) | 30-day treatment (%) |
| Normal<sup>a</sup> | 146 ± 10<sup>a</sup> | P < 0.05 | 163 ± 18<sup>a</sup> | P < 0.01 |
| Castrated<sup>a</sup> | 119 ± 7<sup>a</sup> | 31 ± 1<sup>a</sup> |
| Control<sup>c</sup> | 77 ± 1<sup>c</sup> (100) | 72 ± 8<sup>c</sup> (100) | 62 ± 6<sup>c</sup> (100) | 61 ± 6<sup>c</sup> (100) |
| PPE 0.1<sup>c</sup> | 70 ± 6<sup>c</sup> (91) | 81 ± 6<sup>c</sup> (113) | 62 ± 5<sup>c</sup> (100) | 64 ± 6<sup>c</sup> (105) |
| 0.3 | 68 ± 5<sup>c</sup> (88) | 68 ± 5<sup>c</sup> (94) | 58 ± 9<sup>c</sup> (94) | 57 ± 10<sup>c</sup> (93) |
| 1.0 | 70 ± 4<sup>c</sup> (91) | 74 ± 9<sup>c</sup> (103) | 48 ± 5<sup>c</sup> (77) | 55 ± 9<sup>c</sup> (90) |
| 3.0 | 62 ± 3<sup>c</sup> (81)<sup>c</sup> | 42 ± 6<sup>c</sup> (88)<sup>c</sup> | 38 ± 4<sup>c</sup> (61)<sup>c</sup> | 34 ± 8<sup>c</sup> (56)<sup>c</sup> |
| PE 100 | 57 ± 5<sup>c</sup> (74)<sup>c</sup> | 72 ± 5<sup>c</sup> (100) | 30 ± 1<sup>c</sup> (48)<sup>c</sup> | 60 ± 7<sup>c</sup> (98) |

<sup>a</sup>: Measured 8 days after the castration (N = 9 animals).  <sup>b</sup>: Treated with testosterone (0.15 mg/kg/day, s.c.) alone for 15 or 30 days starting 8 days after the castration (N = 4 or 5 animals).  <sup>c</sup>: PPE or PE was given p.o. in combination with testosterone for 15 or 30 days (N = 4 or 5 animals).  <sup>x</sup>: Statistically significant difference from the castrated rats at P < 0.05 and at P < 0.01, respectively.  <sup>y</sup>: Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.
7 μg/100 mg tissue weight in normal and castrated rats, respectively). Therefore, the effect of castration on the amount of citric acid per 100 mg tissue weight in the prostate was not obvious. The castration, however, decreased the citric acid contents per 100 g body weight in the dorsal lobe (65 ± 7 and 41 ± 3 μg/100 g body weight in normal and castrated rats, respectively) and ventral lobe (163 ± 18 and 31 ± 1 μg/100 g body weight in normal and castrated rats, respectively).

The castration, however, decreased the citric acid contents per 100 g body weight in the dorsal lobe (65 ± 7 and 41 ± 3 μg/100 g body weight in normal and castrated rats, respectively) and ventral lobe (163 ± 18 and 31 ± 1 μg/100 g body weight in normal and castrated rats, respectively).

The testosterone treatment decreased the citric acid amount per 100 mg tissue weight of the dorsal and ventral lobes in castrated rats, and it recovered the amount per 100 g body weight.

The 15-day and 30-day combined treatment of PPE did not affect the content per 100 mg tissue weight in the dorsal lobe. However, PPE decreased the citric acid content per 100 g body weight in the dorsal lobe by the 15-day treatment with higher doses (1.0 and 3.0 mg/kg), and it showed a tendency to decrease it by the 30-day treatment with every dose. PE decreased or showed a tendency to decrease the content both per 100 mg tissue weight and per 100 g body weight in the 15-day and 30-day treatments.

The citric acid content per 100 mg tissue weight in the ventral lobes decreased by the 15-day and 30-day treatments with PPE only in a dose of 3.0 mg/kg. The content per 100 g body weight in the ventral lobe decreased dose-dependently by the 15-day treatment with PPE and also by the 30-day treatment with 3.0 mg/kg of PPE. The 15-day treatment with PE suppressed the contents per 100 mg tissue weight and that per 100 g body weight.

Isocitrate dehydrogenase activity in prostate: Table 10 shows that the castration increased the activity of the enzyme per 100 mg tissue weight and decreased the activity per 100 g body weight in the dorsal and ventral lobes of the prostate 8 days after.

Testosterone treatment recovered the elevated activity per 100 mg tissue weight and the decreased activity per 100 g body weight in both lobes of the castrated rats.

As described in the Introduction, it is known that the hypertrophic prostate has a high citric acid content. This might be associated with the abnormality of enzymes of the TCA cycle in hypertrophic prostate. Previously, we (16) reported that administration of PE to normal rats had no appreciable influence on the prostate weight, but caused significant increases of O₂ uptake and aconitase and isocitrate dehydrogenase activities, without any significant decrease of citric acid content.

First in the present paper, we examined the effect of PPE on the weight of accessory sexual organs as well as citric acid content, O₂ uptake, aconitase and isocitrate dehydrogenase activities of the prostate in normal rats. The O₂ uptake was lower and the citric acid content and isocitrate dehydrogenase activity were higher in the dorsal lobe of the prostate than in the ventral lobe in levels per unit tissue weight. The aconitase activities were almost the same in the dorsal and ventral lobes. As shown in the experiment using the castrated rats, the RNA content was two-fold lower in the dorsal lobe compared with that in the ventral lobe in normal rats in spite of the fact that both lobes almost had the same content of DNA in terms of the amount per unit tissue weight. These results suggest that metabolic activity in the dorsal lobe is lower than that in the ventral lobe.

PPE given to normal rats had little or no effect on the activity per 100 mg tissue weight in the dorsal and ventral lobes by the 15-day and 30-day treatments, but decreased the activity per 100 g body weight in the dorsal lobe by the 30-day treatment.

DISCUSSION

PPE and PE had little effect on the activity per 100 mg tissue weight in the dorsal and ventral lobes by the 15-day and 30-day treatments, but decreased the activity per 100 g body weight in the dorsal lobe by the 30-day treatment.
Table 10. Effect of purified prostate extract (PPE) and prostate extract (PE) on isocitrate dehydrogenase activity in the prostate of castrated and testosterone-treated rats

| Dorsal lobe | Unit/100 mg tissue weight | Unit/100 g body weight |
|-------------|---------------------------|------------------------|
| Dose (mg/kg) | 15-day treatment (%) | 30-day treatment (%) | 15-day treatment (%) | 30-day treatment (%) |
| Normal* | 659 ± 27 | P < 0.01 | 634 ± 69 | P < 0.05 |
| Castrated* | 833 ± 32 | | 371 ± 52 | |
| Control** | 704 ± 40* | (100) | 735 ± 8* | (100) |
| PPE 0.1** | 760 ± 12 (108) | 770 ± 16 (105) | 444 ± 17 (100) | 503 ± 34 (100) |
| 0.3 | 718 ± 22 (102) | 801 ± 20 (109)* | 374 ± 54 (84) | 418 ± 34 (83) |
| 1.0 | 727 ± 4 (103) | 787 ± 19 (107) | 467 ± 83 (105) | 419 ± 31 (83) |
| 3.0 | 709 ± 25 (101) | 779 ± 17 (106)* | 344 ± 40 (77) | 396 ± 38 (79) |
| PE 100 | 708 ± 32 (101) | 793 ± 14 (108) | 355 ± 31 (80)* | 385 ± 29 (77)* |

| Ventral lobe | Unit/100 mg tissue weight | Unit/100 g body weight |
|-------------|---------------------------|------------------------|
| Dose (mg/kg) | 15-day treatment (%) | 30-day treatment (%) | 15-day treatment (%) | 30-day treatment (%) |
| Normal* | 373 ± 36 | P < 0.05 | 393 ± 26 | P < 0.01 |
| Castrated* | 477 ± 8 | | 109 ± 16 | |
| Control** | 379 ± 37* | (100) | 433 ± 24 (100) | 281 ± 24* | (100) |
| PPE 0.1** | 452 ± 35 (119) | 416 ± 20 (96) | 248 ± 25 (88) | 330 ± 24 (89) |
| 0.3 | 457 ± 28 (121) | 441 ± 23 (102) | 373 ± 24 (133)* | 355 ± 31 (96) |
| 1.0 | 412 ± 25 (109) | 447 ± 49 (103) | 343 ± 61 (122) | 327 ± 45 (88) |
| 3.0 | 462 ± 38 (122) | 451 ± 32 (104) | 301 ± 17 (107) | 345 ± 55 (93) |
| PE 100 | 489 ± 12 (129)* | 377 ± 6 (87) | 387 ± 48 (137) | 311 ± 24 (84) |

* Measured 8 days after the castration (N = 4 animals). **: Treated with testosterone (0.15 mg/kg/day, s.c.) alone for 15 or 30 days starting 8 days after the castration (N = 4 animals). ***: PPE or PE was given p.o. in combination with testosterone for 15 or 30 days (N = 4 animals). ±, ±: Statistically significant difference from the castrated rats at P < 0.05 and at P < 0.01, respectively. *:* Statistically significant difference from the control at P < 0.05 and P < 0.01, respectively.

It is well known that weights of the prostate, seminal vesicle and levator ani muscle are increased by administration of an androgenic substance. The increase might be attributed to its androgenic/anabolic activity. It has been also reported that androgens cause elevation of fructose and citric acid contents (10) and acid phosphatase activity (18) in the prostate. On the other hand, it has been documented (13) that accessory sexual organ weights of the prostate and seminal vesicle in rats decrease rapidly by day 8 after castration and show no recovery after that. Also, Nyden and Williams-Ashman (19) and Rudolph and Starnes (13) have reported that the O2 uptake of prostatic tissue in castrated animals is strictly lower than that of intact animals, and the administration of testosterone to castrated
Ones restores the suppressed O\textsubscript{2} uptake of the prostate as well as the decreased weight of accessory sexual organs. In these reports, relatively high doses of testosterone (0.5–1.0 mg/kg/day) were used, and the recovery of prostate weight in castrated rats was found rapidly within 20 days. However, our pilot study indicated that a short term administration of an high dose of testosterone (1.0 mg/kg/day, 12 days) made the prostate of castrated rats large but watery, suggesting the functionally abnormal recovery of the prostate. In the present study, therefore, we used a testosterone dose that would enable the recovery of prostate weight to the normal level after a long term treatment of 30 days, expecting the functional recovery of the prostate to be as normal as possible. This experimental condition allowed a long term combined treatment with PPE, as was desirable to examine the effect of PPE on the recovery of prostate.

We examined the effect of PPE on the weights of accessory sexual organs, DNA and RNA contents, citric acid content and isocitrate dehydrogenase activity of the prostate in castrated and testosterone-treated rats. PPE prevented the testosterone-induced recovery of the prostate and other accessory sexual organ weights, especially that of the dorsal lobe of the prostate. As summarized in Table 11, the castration increased the DNA content and isocitrate dehydrogenase activity per unit tissue weight of the dorsal and ventral lobes, but it decreased RNA contents per unit tissue weight, suggesting that castration suppressed the metabolism in the prostate. It is obvious that testosterone recovered the suppressed metabolism.

PPE prevented the recovery of the parameters per unit body weight associated with

| Table 11. Summary of the effects of purified prostate extract (PPE) on biochemical parameters of the prostate in castrated rats |
|-----------------------------------------------|--------------|----------------|----------------|
| Treatment of castrated rats                  | Per tissue weight | Per body weight |
| Testosterone PPE                             |              |                |
| DNA                                            | Increase\textsuperscript{a} | Decrease\textsuperscript{a} |
| + –                                             | Recovered     | Recovered      |
| + +                                             | NS            | S ++           |
| RNA                                            | Decrease\textsuperscript{a} | Decrease\textsuperscript{a} |
| + –                                             | Recovered     | Recovered      |
| + +                                             | NS            | S ++           |
| Citric acid                                    | Not obvious change\textsuperscript{a} | Decrease\textsuperscript{a} |
| + –                                             | Decreased     | Recovered      |
| + +                                             | NS to S +     | S + to S ++    |
| ISDH                                           | Increase\textsuperscript{a} | Decrease\textsuperscript{a} |
| + –                                             | Recovered     | Recovered      |
| + +                                             | NS            | NS to S +      |

\textsuperscript{a}: In comparison with normal rats. ISDH: Isocitrate dehydrogenase. NS: No suppression compared with the castrated and testosterone-treated rats. S +: Tendency of suppression. S ++: Obvious suppression. S +++: Significant suppression.
the prevention of the recovery in the tissue weight. This effect of PPE was probably due to its ability to suppress the excessive testosterone-uptake into the prostate as evidenced in the preceding paper (7). PPE is a peptide (7), and the agent should display anti-androgenic activity through a mechanism different from that of a steroid hormone such as gestagen. However, PPE did not affect so much the testosterone-induced recovery of the biological parameters per unit tissue weight except for the citric acid content. The reason was unclear why PPE did not suppress the recovery of the parameters per unit tissue weight though it prevented the recovery of the parameters per unit body weight. PPE might not affect the functional recovery of the prostate by testosterone.

PPE reduced the citric acid content per unit tissue weight as well as that per unit body weight in both normal rats and castrated and testosterone-treated rats. It is possible that PPE reduces the citric acid content through some mechanisms other than suppression of testosterone uptake into the prostate. Further investigation is needed about the mechanisms of PPE-induced reduction of citric acid content as well as the role of this activity in the therapeutic efficacy of PE and/or PPE on benign prostatic hypertrophy in which high citric acid content is demonstrated.

The results shown in the present paper suggest that PPE is one of the active principles of PE in its therapeutic efficiency on prostatic hypertrophy. It remains to be investigated whether the activity of PPE is due to the whole molecule of PPE or its fragment produced by digestion in the gastrointestinal tract, because PPE is a high molecular peptide and effective by oral administration.

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