Effects of 2,5-Dimethylpyrazine on Plasma Testosterone and Polyamines- and Acid Phosphatase-Levels in the Rat Prostate

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The effects of 2,5-dimethylpyrazine (DMP) on plasma testosterone and levels of polyamines and acid phosphatase in the prostate of rat were studied. A high dose of DMP administered once daily for two weeks to juvenile male rats significantly decreased plasma testosterone and levels of polyamines and acid phosphatase in the prostate. These effects were not obtained by administration to mature male rats, and the inhibition was thus recognized to occur during the growth period. These findings suggest that a high dose of DMP inhibits the biosynthesis of polyamines and acid phosphatase in the prostate by decreasing the circulating testosterone level.

Keywords 2,5-dimethylpyrazine; plasma testosterone; polyamine; acid phosphatase

One of the alkylpyrazine derivatives, 2,5-dimethylpyrazine (DMP), has been shown to have a delaying effect on the onset of puberty in rats and mice. Recent studies by Novotony *et al.*¹⁾ and by us²⁾ showed that DMP was a physiological urinary element in mice and that it delayed vaginal opening in female rats. Further, our previous findings^{3,4)} suggest that DMP has a suppressive effect on accessory reproductive organ weight in male and female rats, thus making it of interest in the fields of reproductive and endocrinological pharmacology. In this study, we examined the effect of DMP on plasma testosterone level and polyamines and acid phosphatase levels in the prostate of rats.

MATERIALS AND METHODS

Four- and six-week-old male Wistar strain rats (Saitama Experimental Animals Supply Co., Saitama, Japan) were obtained. The rats were housed in a controlled environment ($22 \pm 1^\circ\text{C}$, $53 \pm 3\%$ humidity, lights on from 6:30 to 18:30) and food and water were available *ad libitum*. DMP was purchased from Aldrich (Milwaukee, Wisconsin, U.S.A.) and dissolved in 0.9% NaCl solution. The doses given to four-week old rats were 10, 30, 70, 100 and 300 mg/kg s.c. once a day for 2 weeks. Six-week old animals were administered two doses of 100 and 300 mg/kg. Control rats received saline *via* the same route and on the same schedule.

Measurement of Testosterone Level At 18 h after the final administration of DMP, the rats were decapitated, and blood samples were collected from the trunk into heparin-treated tubes. Plasma specimens, prepared by centrifugation at 2800 rpm for 15 min, were stored at -20°C until used for determination of testosterone levels; this was accomplished by radioimmunoassay⁵⁾ (SRL Laboratory, Hachioji, Tokyo).

Determination of Polyamines in the Prostate Polyamines, spermidine and spermine were determined by high-performance liquid chromatography (HPLC).^{6,7)} After weighing the prostate, about 100 mg of tissue was homogenized in 1 ml of cold 0.3 M perchloric acid in the presence of a known amount of internal standard, using a Potter-Elvehjem homogenizer. The homogenate was

centrifuged at 2800 rpm for 15 min. The clear supernatant obtained after centrifugation of the perchloric acid-treated sample was frozen (-20°C) until further analysis. In glass tubes (1.5×10.5 cm), 2 ml of 2 M sodium hydroxide and 10 μl of benzoyl chloride were added to 10 μl of the perchloric acid supernatant. Tubes were mixed vigorously and incubated for 30 min at room temperature under gentle rotation. Subsequently, 1 ml of saturated sodium chloride solution and 3 ml of diethylether were added and the tubes were mixed and gently rotated for 30 min. After centrifugation, the ether phase was removed, washed with 2 ml of water, centrifuged and transferred to another tube. The solvent was evaporated under a stream of nitrogen gas at 38°C and the residue was resuspended in 200 μl of methanol. Then, 10 μl of the methanol solution was injected onto the HPLC column. Polyamines were determined by the ratio of their area relative to 1,8-diaminooctane.

HPLC and Conditions A SSC-3100K (Senshu-Kagaku, Tokyo) high-performance liquid chromatograph equipped with a SSC-300B UV spectrophotometer (Senshu-Kagaku, Tokyo) was used. The ultraviolet detector was set at 254 nm. The chromatograms were recorded with a C-R6A Chromatopac (Shimadzu, Tokyo). Standard spermidine and spermine, and 1,8-diaminooctane used as an internal standard were purchased from Sigma Chemical Co., (St. Louis, Mo. U.S.A.) and Aldrich Chemical Co., (Milwaukee, Wisconsin, U.S.A.). 1,8-Diaminooctane (10 mg/ml, 10% HCl) was used as an internal standard and the calculated dose was added in a volume of 10 μl . All other reagents were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and were of analytical reagent grade for HPLC. Acetonitrile and distilled water were degassed prior to use. Precolumn: Senshu Pak ODS-1031-N, 5 μm , 4.6 mm i.d. $\times$ 30 mm, main column: Senshu Pak ODS-UH-1251, 5 μm , 4.6 mm i.d. $\times$ 250 mm, mobile phase: $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (55:45 (v/v)), flow rate: 0.8 ml/min.

Determination of Acid Phosphatase Level and Protein Acid phosphatase level in the prostate was determined with a Wako-K Test kit (Wako Pure Chemical Industries, Ltd.). Protein was determined by the method of Lowry *et al.*⁸⁾

Statistics Statistical significance was evaluated by

TABLE I. Effect of DMP on Polyamines and Acid Phosphatase Levels in the Prostate and Plasma Testosterone in Juvenile Male Rats

DMP (mg/kg)	Spermidine (μ mol/g)	Spermine (μ mol/g)	Acid phosphatase (KA unit/mg protein)	Plasma testosterone (ng/ml)
0	29.41 $\pm$ 3.65	8.13 $\pm$ 0.76	78.2 $\pm$ 6.86	1.84 $\pm$ 0.39
10	32.05 $\pm$ 4.16	8.94 $\pm$ 1.16	81.9 $\pm$ 5.03	2.05 $\pm$ 0.11
30	26.80 $\pm$ 1.81	7.50 $\pm$ 0.87	69.0 $\pm$ 5.13	1.80 $\pm$ 0.25
70	20.32 $\pm$ 4.22	4.91 $\pm$ 1.77 ^{a)}	60.9 $\pm$ 7.22	0.91 $\pm$ 0.08 ^{a)}
100	20.76 $\pm$ 1.08 ^{a)}	4.67 $\pm$ 1.27 ^{a)}	61.6 $\pm$ 2.46 ^{a)}	0.72 $\pm$ 0.10 ^{a)}
300	18.52 $\pm$ 2.19 ^{b)}	3.88 $\pm$ 0.96 ^{b)}	56.2 $\pm$ 3.27 ^{a)}	0.60 $\pm$ 0.10 ^{a)}

Each value represents the mean $\pm$ S.E. of five rats. KA unit: Karmen unit. a) Significantly different from the control value at $p < 0.05$. b) Significantly different from the control value at $p < 0.01$.

TABLE II. Effect of DMP on Polyamines and Acid Phosphatase Levels in the Prostate and Plasma Testosterone in Mature Male Rats

DMP (mg/kg)	Spermidine (μ mol/g)	Spermine (μ mol/g)	Acid phosphatase (KA unit/mg protein)	Plasma testosterone (ng/ml)
0	37.46 $\pm$ 12.52	11.84 $\pm$ 3.64	81.5 $\pm$ 6.04	2.42 $\pm$ 0.64
100	35.71 $\pm$ 7.81	11.03 $\pm$ 1.58	79.8 $\pm$ 4.50	1.96 $\pm$ 0.32
300	30.62 $\pm$ 11.67	9.86 $\pm$ 4.19	75.6 $\pm$ 6.42	1.80 $\pm$ 0.41

Each value represents the mean $\pm$ S.E. of five rats. KA unit: Karmen unit.

Student's *t*-test.

RESULTS AND DISCUSSION

After 14 administrations of DMP to juvenile rats (4-week-old), polyamine and acid phosphatase levels in the prostate and plasma testosterone were significantly decreased by 14 administrations of a high dose of DMP compared with saline-treated rats (Table I). On the other hand, the levels of these markers were not influenced by administration for 3 or 7 d (data not shown). DMP also had no effect on mature (6-week-old) rats at the dose of 100 mg/kg. With administration of 300 mg/kg, these markers were slightly lower than with the administration of 100 mg/kg (Table II).

Acid phosphatase level, arginase activity and polyamine level in human and rat prostate have been considered as indexes of testosterone function.^{9,10} In humans and rats, the levels of polyamines and acid phosphatase are higher in the prostate than in other organs, and polyamines are thought to be cell proliferation factors in the prostate.¹¹ Furthermore, the levels of these markers in the prostate are dependent upon adequate levels of circulating testosterone. DMP may have an inhibitory effect on the growth period in immature animals, thus leading to the decrease in testosterone level. On the other hand, effect of the metabolite (*ex. carboxylic acid derivative*) of DMP on the gonadal function is not yet known.¹²

The roles of these polyamines and acid phosphatase in reproductive physiology have been reviewed by Williams-Ashman and Lockwood¹³ and by Ablin.¹⁴ Several researchers¹⁵⁻¹⁷ reported that the reduced polyamine levels in the castrated rat prostate and seminal vesicles

were restored after testosterone administration. Accordingly, the relationship between the levels of polyamines and acid phosphatase in the accessory reproductive organs and the androgenic effects of testosterone seems clear. The present findings suggest that DMP inhibits polyamine synthesis and acid phosphatase levels by lowering the plasma testosterone level in juvenile male rats.

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