Effects of Pantothenic Acid on Testicular Function in Male Rats

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ABSTRACT. Pantothenic acid (PaA) is a water-soluble vitamin required to sustain various physiological functions in animals. The physiological roles of PaA on testicular function, in particular, testicular endocrinology and sperm motility, were investigated in rats. Male rats at 3 weeks of age were fed a PaA-free diet or a 0.0016% PaA diet (control) for 7 weeks. Total body weight, as well as the weights of the liver, kidney, pituitary, testis, epididymis, seminal vesicle and prostate; sperm motility; and the plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and corticosterone were measured in rats at 10 weeks of age. Body weight gain decreased from 5 weeks of age in rats fed the PaA-free diet compared with the control. The relative weights of the testes were significantly higher in the PaA-deficient group compared with the control group. Several parameters of sperm motility were significantly reduced in the PaA-deficient group compared with the control group. In addition, the plasma concentrations of testosterone and corticosterone were significantly lower in the PaA-deficient group compared with the control group, whereas the plasma concentrations of FSH and LH showed no change. These results clearly demonstrate that PaA is an essential factor in testicular endocrinology and sperm motility in male rats.

KEY WORDS: pantothenic acid, sperm motility, testosterone.

FULL PAPER

Pantothenic acid (PaA), one of the water-soluble B-complex vitamins, was discovered by Williams et al. in 1933 as the growth factor of “Bios” yeast [36]. It is essential for various physiological functions and is a component of coenzyme A (CoA) [17, 23]. In the body, PaA is converted to CoA through binding with ATP and L-cysteine [6]. The chemical reactions that generate energy from food require CoA, and CoA in its acetyl form is the sole energy source of the tricarboxylic acid cycle. CoA is also required for synthesis and metabolism of cholesterol and production of steroid hormones, long-chain fatty acids, prostaglandins and the neurotransmitter acetylcholine; it is vital not only for energy metabolism but also for numerous physiological activities [33]. It is also involved in the synthesis of adrenal cortical hormones [11, 12, 27, 28, 37] and growth of skin, hair and nerves [8, 16]. PaA was recently reported to protect cells and organs against peroxidative damage by increasing the content of cell glutathione [31].

PaA is known to play an important role in the male and female reproductive systems. Its deficiency in the diet of female rats leads to abnormal development of the ovary and uterus, and some treated animals even show abnormalities in the estrous cycle [20, 21]. Jukes and Nelson et al. demonstrated increased implantation failure or resorptions in a group of stock rats placed on PaA-deficient diets at mating [13, 22]. Sure noted that reproduction was abnormal when animals were put on purified diets without the addition of calcium pantothenate, which resulted in either “sterility” of the mother or a high incidence of stillbirths [32]. While a number of studies have reported on the effects of PaA on the female reproductive system [13, 20–22, 32], only a few have looked at the effects on the male reproductive tract.

Ashburn described some changes in the testes of pantothenic acid-deficient rats, namely large multinuclear or abnormal spermatids. In several experiments conducted in this laboratory, pantothenic acid-deficient animals were observed to have a damaged germinal epithelium and impaired spermiogenesis [2–5, 16, 24]. However, there is no evidence regarding the effects of PaA on the development of testis function in rats. In the present study, the effects of PaA-deficiency on epididymal sperm motility and the levels of testosterone, corticosterone and gonadotropins were investigated during the developing period in male rats.

MATERIALS AND METHODS

Chemicals: Vitamin-free milk casein, sucrose and L-methionine were purchased from Wako Pure Chemical Industries (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, the mineral mixture (AIN-93M) [30] and the vitamin mixture (AIN-93VX containing 25% choline bitartrate) [30] were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Animals: Male Wistar-Imamichi rats, 3 weeks of age, from the Imamichi Institute for Animal Reproduction (Kasumigaura, Ibaraki, Japan) were used in this study. They
were kept in the animal room under standard housing conditions with controlled lighting (lights on 05.00 hr–19.00 hr), temperature (23 ± 2°C) and humidity (50 ± 10%).

The rats were randomly divided into two groups, each group containing fifteen rats. In the first group, the rats were fed a PaA-free, 20% casein diet, and the other group was fed the same diet but with the addition of 0.0016% PaA-Ca (Table 1). The group fed the diet containing 0.0016% PaA was considered the control group. The special PaA diet was fed to the rats ad libitum for 7 weeks. The rats were allowed free access to diet and tap water.

All experimental procedures were performed in accordance with the requirements established under the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology.

**Sample collection:** The rats were sacrificed by decapitation at the end of the seventh week. Blood samples were collected and centrifuged at 1700 × g at 4°C for 30 min. The plasma was separated and stored at –20°C until hormonal analyses. The testes, epididymides, seminal vesicles, prostate, liver, kidney and pituitary were removed, cleared of adhering connective tissue and weighed.

**Computer-assisted sperm motility analyses:** Semen from the cauda epididymides was collected in a 1.5 ml-sample from the cauda epididymides was diluted with 1 ml Tyrode’s medium (a 3 μl semen sample from the cauda epididymides was diluted with 1 ml Tyrode’s medium). The sperm motility was measured by computer-assisted sperm analysis (CASA) using the C. IMAGING C. MEN system (C. IMAGING systems, Compix Inc., Tualatin, OR, U.S.A.). Briefly, the diluted sperm suspension was placed in prewarmed slide chambers at a depth of 20 μm. The slides were viewed using an Olympus microscope (Olympus BX50F, Olympus Optical Co., Ltd., Tokyo, Japan) connected to a personal computer. The temperature of the microscope stage was maintained at 37°C throughout the observation by a stage warmer (MP-10DM, Kitazato Supply Co., Ltd., Tokyo, Japan). CASA was performed using the C. IMAGING C. MEN system and the C. IMAGING software (C. IMAGING systems, Compix Inc., Tualatin, OR, U.S.A.) [25]. Our CASA system was based upon the analysis of 15 consecutive, digitized photographic images obtained from a single field that were taken using a time-lapse of 0.5 sec. Two to three separate fields were taken for each sample. The percentage of motile spermatozoa (%), straight-line velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), linearity index (ratio of the straight line distance to the actual tracked distance), deviation of the sperm head from the mean trajectory (ALH, mean μm), maximal amplitude of lateral head displacement (ALH, max μm) and beat frequency of centroids crossing the average trajectory (BCF, Hz) were determined.

**Measurement of hormone:** Plasma concentrations of testosterone [35] and corticosterone [14] were determined using a double-antibody radioimmunoassay system with 125I-labeled radioligand, as described previously. The intra- and interassay coefficients of variation were 12.80% and 13.60% for testosterone and 5.10% and 16.04% for corticosterone, respectively.

Plasma concentrations of FSH and LH were measured using the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) RIA kits for rat FSH and LH. The iodinated preparations were rat FSH (I-7) and LH (I-7). The antisera used were anti-rat FSH (S-11) and LH (S-10). Results were expressed in terms of NIDDK rat FSH (RP-2) and LH (RP-2). The intra- and interassay coefficients of variation were 2.94% and 12.82% for FSH and 7.90% and 18.11% for LH, respectively.

**Statistical analyses:** All results were expressed as mean ± standard errors of the mean (SEM). Statistical comparisons between the two groups were performed by Student’s t-test when uniformity of variance was confirmed by the F-test. P<0.05 was considered to be statistically significant.

### RESULTS

**Effects of PaA deficiency on body weight gain:** Body weight gain was compared between rats fed the control and 0% PaA diets for 7 weeks (Fig. 1). Body weight increased in both groups. However, the body weight in the 0% PaA diet group was significantly lower than that in the control group (Student’s t-test).

![Fig. 1. Effects of PaA deficiency on body weight gain in male rats fed a control diet (○) and those fed a 0% PaA diet (▲). The values are expressed as means ± SEM (n=15). * P<0.05 indicates a significant difference between the experimental and control groups (Student’s t-test).](image-url)
group was significantly lower compared with the control group from 2 weeks of treatment onward.

Effects of PaA deficiency on tissue weights: The tissue weights of the rats fed the control and 0% PaA diets are shown in Table 2. All tissue weights in the 0% PaA group were significantly lower than in the control group. When the values were expressed as mg/100 g of rat body weight in Table 3, the weights of the liver and kidney were not significantly different between the two groups. However, the testis weight was significantly higher in the 0% PaA group compared with the control group. The weights of the epididymis and seminal vesicle, prostate and pituitary tissues were not significantly different.

Effects of PaA deficiency on sperm motility: Almost all parameters of sperm motility were significantly (p<0.05) lower in the 0% PaA group compared with the control group (Fig. 2). The linearity index and ALH mean were lower in the 0% PaA group compared with the control group, but there were no significant differences. Sperm head count was not significantly different between the two groups (not shown).

Effects of PaA deficiency on secretion of LH, FSH, testosterone and corticosterone: There were no significant differences in the plasma concentrations of FSH and LH (Fig. 3). However, the plasma concentrations of testosterone (Fig. 3) and corticosterone (Fig. 4) were significantly lower in the 0% PaA group compared with the control group.

DISCUSSION

The present study clearly demonstrates that the two essential testicular functions, secretion of testosterone and sperm quality, are reduced in PaA-deficient animals. The plasma concentrations of testosterone were significantly lower in the PaA-deficient rats compared with the control rats, although there were no differences in the concentrations of plasma LH and FSH between the two groups. These results suggest that PaA acts directly on the testis but does not affect the hypothalamus or pituitary levels. In addition, sperm motility and most sperm motility parameters were significantly reduced in the PaA-deficient animals compared with the control group. These results clearly demonstrate that PaA is an essential factor in the testicular function of rats.

There is evidence that synthesis of cholesterol is impaired in PaA-deficient animals [19]. Since synthesis of steroid hormones starts from cholesterol [1, 7, 10, 15], the decrease in circulating testosterone in PaA-deficient animals probably results from a decrease in cholesterol synthesis induced by the PaA deficiency. This is also true for steroid hormone synthesis in the adrenal gland. The present study clearly showed a decrease in circulating corticosterone in PaA-deficient rats. Consistent with the present study, our previous work demonstrated that adrenal cells in PaA-treated male rats exhibited higher basal levels of corticosterone than those in control rats [12], and the response of ACTH and/or
prolactin on corticosterone and progesterone release was higher in the PaA-treated male rats than in control rats. The present study clearly demonstrated that PaA is an essential factor for maintenance of testicular and adrenal functions in rats, in agreement with the previous study.

In addition, the decline in testosterone secretion leads to a reduction in sperm quality in PaA-deficient rats. It is well-known that testosterone is essential for spermatogenesis, acting at several steps in the pathway. Testosterone is indispensable in the elongation of round spermatids. When testosterone secretion is decreased experimentally by removal of the pituitary gland, spermatocytal and spermatidal denaturation occurs, and there is a decrease in sperm number [29]. In previous studies, it has been reported that as the plasma concentration of testosterone decreases, sperm motility also decreases [9, 18]. However, sperm motility does not decrease immediately, but rather decreases gradually along with the concentration of plasma testosterone [26]. In the present study, testosterone-dependent sperm motility parameters significantly decreased in rats fed a PaA-deficient diet as compared with control rats, which is correlated with the lowering of testosterone secretion by PaA deficiency.

In this study, the circulating levels of LH and FSH in the
PaA-deficient rats did not increase, although the circulating levels of testosterone decreased. The reason why the levels of LH and FSH remained unchanged is currently unknown. Our previous report demonstrated that corticotrophin-releasing hormone (CRH) and \( \beta \)-endorphin have inhibitory effects on gonadotropin-releasing hormone secreted from the hypothalamus, which, in turn, results in reduced secretion of LH and FSH from the pituitary gland [34]. In the present study, the basal levels of corticosterone declined in the PaA-deficient rats, indicating that the negative feedback effect of corticosterone on the secretion of CRH was also reduced. Therefore, these results suggest that the hypersecretion of CRH and \( \beta \)-endorphin may suppress secretion of LH and FSH from the pituitary gland.

Although the absolute weight of the testis was significantly low in the PaA-deficient group as compared with the control group, the relative weight of the testis was significantly higher in the PaA-deficient group. The reason why the testicular weight is different by body weight is unclear. Supporting the present findings, a previous paper reported similar results for the changes in testicular weight in the PaA-deficient rat (Shibata K. et al.) [30].

In conclusion, the present study clearly demonstrated that a diet deficient in PaA induced abnormalities of testicular function and spermatogenesis in rats. Moreover, they suggest that deficiency in PaA may be primarily mediated by the decrease in endogenous production of cholesterol in the testes and adrenal gland.

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