

EFFECT OF ALPHA-BLOCKERS ON EPIDIDYMAL SPERM CONCENTRATION, MOTILITY AND TESTICULAR PRODUCTIVITY IN THE RAT

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The present study was undertaken to determine whether α -blockers affect epididymal sperm parameters and testicular spermatogenesis.

Sprague-Dawley rats were given orally a 1 mg/kg body weight dose of Bunazosin, a selective α -adrenoreceptor antagonist, daily for one month as a short-term administration group and three months as a long-term administration group. Using a micropuncture technique, epididymal sperm concentration and sperm motility were measured.

Epididymal sperm concentrations were significantly increased in the Bunazosin group, but progressive motility of spermatozoa were not altered. Daily testicular sperm production was also increased after administration of Bunazosin. There were no differences in epididymal sperm concentration and daily sperm production between the one-month group and three-month group.

An epididymal wide storage capacity and short transit time after administration of the α -blocker could increase the sperm concentration in the cauda epididymidis. Because there were no differences in the epididymal sperm parameters and daily testicular sperm production between the two groups, these changes may occur at 1 month after administration of the α -blocker. Moreover, the α -blocker might affect testicular function.

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Key words: α -blocker, Sperm parameter, Epididymis, Testis

INTRODUCTION

While many studies have shown that the contractions of mammalian reproductive tracts are controlled by autonomic nerves^{1–3)}, the effect of autonomic drugs on epididymal sperm parameters and testicular spermatogenesis has not been reported.

The present study was designed to examine whether α -blockers affect epididymal sperm parameters and daily testicular sperm production in the rat.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (400–570 g) were obtained from Charles River Co. Ltd. (Tokyo, Japan). They were maintained in a room with a light/dark period of 12 h/12 h, at a temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of 50%. They were allowed to acclimate after shipment for at least one week before experimental use.

In Vivo Micropuncture Technique. Preparation of micropipettes and the method of micropuncture were as previously described by Hibi et al⁴⁾. Micropipettes for puncture were drawn on a horizontal puller (Narishige Scientific, model PN-3, Tokyo, Japan) from constant-bore flint glass tubing with an outside diameter of 1 mm and an inside diameter of 0.7 mm. The pipette tips were sharpened on a rotating wet stone grinder (Narishige Scientific, model EG-6, Tokyo, Japan) to about 75 μm diameter.

Under intraperitoneal urethan anesthesia, the testis and epididymis were exposed through a scrotal incision and placed in a 35°C testicle warmer. After the exposed epididymis was immobilized in 2% agar, moistened with water-equilibrated mineral oil to prevent surface dehydration and to improve temperature regulation. A micropipette was placed in a micromanipulator (Narishige Scientific, model M-2) and was inserted directly into the epididymal tubules.

Epididymal Sperm Concentrations. Sperm concentrations in the lumen of the epididymidis were determined as previously described⁵⁾. Samples were collected from both epididymal caput and cauda. Briefly, spermatozoa were obtained by *in vivo* micropuncture, put through a microdilution procedure, and counted on a hemocytometer (10 times) by standard techniques.

The "Distance-Traveled" Assay. Progressive sperm motility was quantified as previously described by Turner et al⁶⁾. About 0.5 μl cauda epididymal fluid was collected and diluted with 4.5 μl minimum essential medium (MEM; Gibco Laboratories, Grand Island, NY). A prefilled rectangular 0.05 mm $\times$ 0.5 mm $\times$ 50 mm glass micropipette (Vitro Dynamics, Rockway, New Jersey) was inserted into the sample. The rectangular micropipette was filed with the MEM. After insertion into the diluted epididymal sperm sample, the pipette was rested on a small cork support block at an approximately 20°

angle. The entire apparatus was covered by a black box and incubated for 30 minutes at 37°C. Then the distance traveled by the spermatozoa in the pipette was examined under a light microscope.

Daily Testicular Sperm Production. To examine the effect of α -blockers on testicular spermatogenesis, daily sperm production (sperm/testis g/day) was determined by the method of Amann and Lambiase⁷⁾. Testes were decapsulated, weighed and homogenized in 50 mL 0.154 M NaCl containing 0.5% (vol/vol) Triton X-100, and 0.02% sodium azide. Condensed sperm nuclei in the resultant solution were counted on a hemocytometer, and sperm/g testis was determined. These values were divided by 6.1 days to convert them to sperm/g/day.

Experiments. We used Bunazosin (Eisai Co. Ltd., Tokyo) as the α -adrenoceptor antagonist. Bunazosin was dissolved in distilled water. Two different groups of rats received bunazosin at a daily dose of 1 mg/kg for one month as short-term administration and three months as long-term administration. A dose of 1 mg/kg of Bunazosin for the rat was equivalent to the clinically used dosage of this drug for hypertensive human patients. The control group animals received distilled water. After completion of Bunazosin administration, epididymal sperm concentration, progressive motility and daily testicular sperm production were determined. Epididymal fluid was obtained from caput and cauda epididymidis three months after initiation of drug treatment to compare the drug response at two different epididymal portions.

Statistical Analysis. Statistical analyses were performed with ANOVA; logarithmic transformations of data were used when appropriate for unequal variances, and Fisher's test was used for post hoc comparisons if $p < 0.05$. All the data were expressed as means $\pm$ the standard error of the mean.

RESULTS

Epididymal Sperm Concentration (at cauda). Cauda epididymal sperm concentrations in the control group were $1.84 \pm 0.12 \times 10^9$ /ml. ($n=18$) These values were significantly increased in the Bunazosin group. (one month group: $2.84 \pm 0.20 \times 10^9$ /ml $n=9$, and three months group: $2.87 \pm 0.19 \times 10^9$ /ml $n=10$, $p < 0.0001$) However, there were no significant differences between the one-month group and three-month group (Fig. 1).

Distance Traveled. Fig. 2 shows the data from the motility assay, representing the distance traveled. There were no significant differences with the group.

Daily Testicular Sperm Production. Daily sperm production was $17.42 \pm 0.79 \times 10^6$ /g/day in the control group ($n=14$). These values were significantly increased in the Bunazosin group (one-month group: $21.08 \pm 0.68 \times 10^6$ /g/day $n=9$, and three-month

Epididymal Sperm Concentration: at Cauda

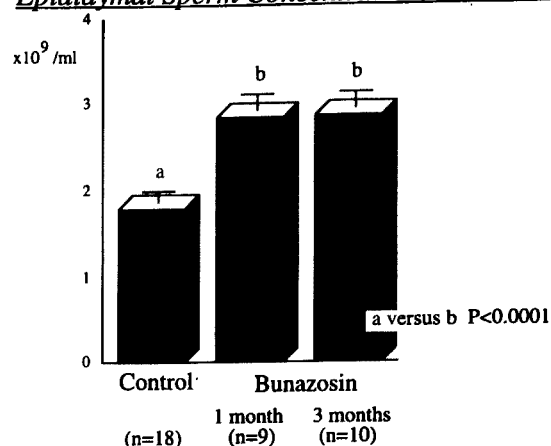


Fig. 1 Cauda epididymal sperm concentration at one month and three month after administration of α -blocker. N represents animal number.

Progressive Motility of Spermatozoa

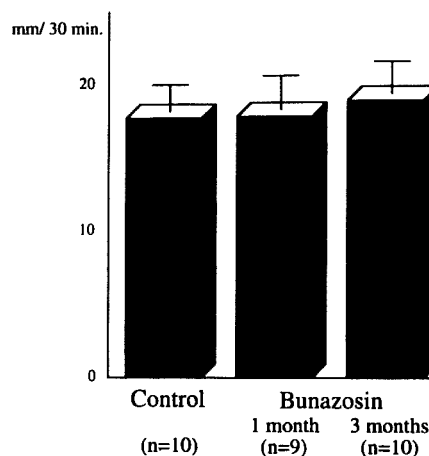


Fig. 2 Progressive motility of cauda epididymal spermatozoa at one month and three months after administration of α -blocker. N represents animal number.

group: $20.51 \pm 0.85 \times 10^6$ /g/day $n=10$, $p < 0.0024$ and $p < 0.028$, respectively). However, there were no significant differences between the one-month group and three-month group (Fig. 3).

Difference of sperm concentration in two retrieved sites of epididymis (caput and cauda) Epididymal sperm concentration of the caput epididymidis ($1.25 \pm 0.09 \times 10^9$ /ml $n=9$) was significantly lower than that of the cauda epididymidis ($1.80 \pm 0.12 \times 10^9$ /ml $n=189$ in the control group ($p < 0.0056$). Nevertheless, no significant differences were noted in the sperm concentration between caput epididymidis and cauda epididymidis after administration of Bunazosin for three months (Fig. 4).

DISCUSSION

The autonomic nervous system has a very

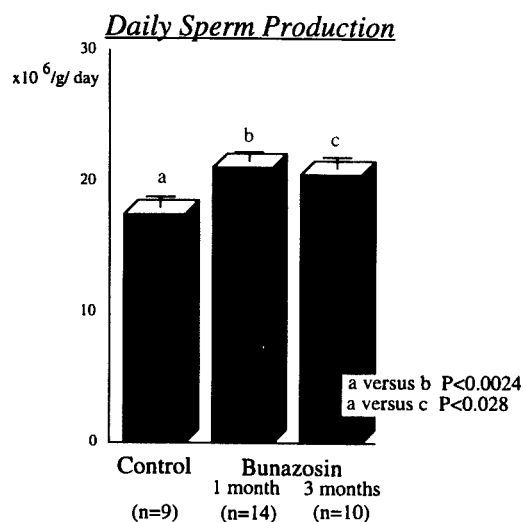


Fig. 3 Daily testicular sperm production at one month and three months after administration of α -blocker. N represents animal number.

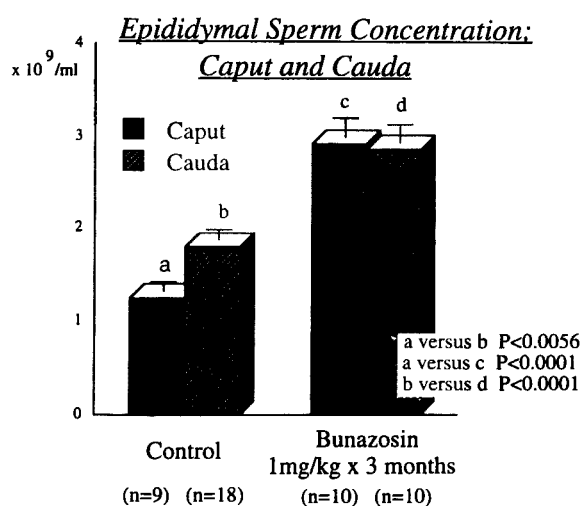


Fig. 4 Differences in sperm concentration between caput and cauda epididymidis at three months after administration of α -blocker. N represents animal number.

important role in maintaining the transport and storage of spermatozoa in the testis and epididymis. We previously reported that α -blockers induced the increase in velocity of tubular fluid flow and short epididymal transit time and subsequently elicits the increase in accumulation of cauda epididymal sperm numbers.⁴⁾

In the present study, there were significant differences in the epididymal sperm concentration between caput and cauda epididymidis in the control group. This might be because the cauda epididymidis is the place of storage of spermatozoa. However, there was no significant difference in the sperm concentration between caput and cauda epididymidis after administration of α -blockers for three months. This may be because α -blockers not

only increase the storage capacity in the cauda epididymidis but also affects testicular function. Moreover, daily testicular sperm production was increased by treatment with α -blockers. These findings still remain unexplained because there is a paucity of α -receptors in the testis⁸⁾.

An important concern is whether this drug can affect sperm maturation in the epididymis and testicular temperature. The epididymal physiology, such as the epithelial transport mechanism or the blood-epididymal barrier, could be potentially changed by α -blockers. This alteration may have a detrimental effect on sperm maturation if α -blockers are administered for a longer period. Increased testicular daily sperm production could be explained by the fact that α -blockers cause relaxation of myoid cells, which leads to dilatation of the seminiferous tubular lumen. Therefore, the action site of α -blockers in the rat reproductive tract for increasing sperm concentration may be both the seminiferous tubules and the epididymal tubules.

In conclusion, α -blockers have significant effects on epididymal sperm concentration and might affect testicular sperm production. Further experimental studies to clarify the effect of α -blocker on testicular blood flow and temperature are needed.

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和文抄録

ラット精巣上体管内液の精子濃度および運動性, 精巣の精子産生能に対する α 遮断剤の効果

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α 遮断剤の精巣上体管内液の質および精巣の精子産生能に対する効果を検討した。SD ラットに α 遮断剤である塩酸ブナゾシンを短期投与群として1カ月, 長期投与群として3カ月, 体重あたり 1 mg/kg 強制経口投与した。精巣上体管内液の採取は微小穿刺法によった。精子濃度は塩酸ブナゾシン投与にて著明に増加したが, 運動性に差はみられなかった。精子の産生能もまた塩酸ブナゾシン投与により増加した。しかし

短期投与群と長期投与群とでは精子濃度, 精子の産生能とも差は認められなかった。精巣上体尾部の精子濃度の増加した理由は, α 遮断剤の投与により増加した精巣上体での精子の貯留スペース, および短くなった精子の精巣上体通過時間, によると考えられた。また α 遮断剤は精巣への影響もあると考えられた。

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