Decrement of blood flow precedes the involution of the ventral prostate in the rat after castration

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Abstract Blood flow to the rat ventral prostate (VP), dorsolateral prostate (DP), and Dunning R3327 prostatic tumors was measured at different times up to 7 days after castration, using the microsphere method. In the VP organ weight was decreased from day 3 onwards. Blood flow was, however, already significantly decreased from day 1. The reduced blood flow in VP in 1-3 and 7-day castrated animals could be reversed by testosterone treatment. Organ weight was slightly decreased but blood flow was unaffected by castration in DP. Castration left Dunning tumor volume and blood flow unaffected. Using immunohistochemistry, androgen receptors were observed in epithelial and stromal cells in VP, DP and Dunning tumors, but not in blood vessels. Castration is known to induce apoptosis in the VP, but not in the DP or in Dunning tumors. This suggests that a reduction in blood flow might be an important component for the castration-induced involution and apoptosis in prostatic tissue. The reason why castration reduces blood flow only in the VP, and not in the DP or Dunning tumor is unknown.

Key words Prostate · Castration · Apoptosis · Blood flow · Radioactive microspheres · Androgen receptors

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Materials and methods

Animals and treatment groups

Male Sprague-Dawley rats were purchased from Mølégaard, Denmark. They were housed under controlled conditions and given water and pellets ad libitum. The experiments were done according to the Swedish Legislation on animal care, and approved by the local ethical committee on animal protection. At the time of the experiments the animals weighed 350–500 g. A total of 77 animals were divided into five groups. Group 1 served as untreated controls and groups 2 to 5 were castrated at different times before blood flow measurements. Rats were anesthetized with a single intramuscular injection of Hypnorm (fluanisomum 10 mg/ml and fentanylum 2 mg/ml) 0.5 ml/kg body weight and the castration was done through a single scrotal incision. The animals were castrated at 12 h, 24 h, 3 days and 7 days before blood flow was measured.

To elucidate whether the observed changes in prostatic blood flow were reversible by testosterone administration or not, three groups of animals were castrated and hormonally substituted with a single subcutaneous injection of testosterone entanate (Testovirone-Depot, Schering, Germany) 250 mg/ml, 0.2 ml 8 or 24 h prior to blood flow measurements. Previous studies have shown that doses smaller than this restore blood flow to the VP in castrated rats [19]. Substitution experiments were performed on 43 animals castrated 1, 3 and 7 days before blood flow was investigated as well as on a control group of untreated animals.

For tumor experiments, 18 Copenhagen X Fisher hybrid male rats of the same size and origin as the Sprague-Dawley rats, were implanted with a 1 x 1 mm piece of the transplantable cell line Dunning R3327-PAP, as previously described [21]. Some of the tumor bearing rats were castrated when the tumors had reached a size of approximately 1 cm³.

Blood flow measurements

Rats were anesthetized with a 1:1:2 mixture of Hypnorm (fluanisomum 10 mg/ml and fentanylum 0.2 mg/ml, Janssen Pharamaceutica), Dormicium (midazolam 5 mg/ml, Roche) and sterile water, 3.0 ml/kg administered as a single intraperitoneal injection. During the experiments, the animals were kept supine on a heated pad.

Blood flow to different organs was measured using radioactive microspheres (diameter 13.5 ± 0.1μm, Dupont Biotechnology, Wilmington, Del.) labeled with 141Ce. The method used was originally described by Rudolf and Heymann [32] and modified by Damber and Janson [14, 15]. Manual vortexing was done directly on the injection syringe. One milliliter of microspheres dissolved in 0.15 M NaCl was injected over 30 s. Aspiration of the reference sample from the tail artery was at least continued 15 s after the end of the injection of spheres. The animal was then killed by thoracotomy.

The VP and DP were dissected out and biopsies were also taken bilaterally from the kidneys. The tumors were dissected from their fibrous capsules. A central and a peripheral part from each tumor were analyzed. When the flow to the distal and proximal parts of the VP was studied, the same method was used, except that the VP was immersion-fixed in 4% formalin in phosphate-buffered saline (PBS) to simplify the dissection, which was done under a dissection microscope. The radioactivity was measured in an automatic gamma counter (Rackgamma, LKB, Sweden). Blood flow is expressed as flow per mass unit and values are given as ml x min⁻¹ x 100 g⁻¹.

Androgen receptor immunohistochemistry

Adult rats were perfusion-fixed with Bouin’s solution for 15 min. Testes, VP, and DP were then removed and immersion-fixed for 120 min in the same fixative, and subsequently dehydrated and embedded in paraffin. Six-micrometer thick sections were mounted on poly-L-lysine-coated slides (Sigma, St. Louis, Mo.). The sections were deparaffinized, rehydrated and heated in a microwave oven (600 W) for 2 x 5 min in citrate buffer using an antigen retrieval method as earlier described [38]. The sections were then incubated overnight at 4°C with rabbit androgen receptor IgG (Biogenesys, N.H.) diluted 1/30. Localization of antibody-antigen complex was performed using the ABC (avidin-biotin complex) technique (Vector Laboratories, Burlingame, Calif.) and peroxidase activity was visualized using AEC (3-amino-9-ethyl-carbazole; Sigma). Prostatic sections of castrated animals and controls, as well as testis sections were examined by light microscopy.

Statistics

Values are expressed as mean ± standard error of the mean (SEM). Comparisons between groups were made using the non-parametric Mann-Whitney U-test with the support of SPSS for Windows statistical program v. 6.1. A P-value less than 0.05 was considered significant.

Results

Blood flow, tumor and organ weights

VP wet weight was not significantly changed until 3 days after castration and there was a marked decrease at 7