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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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Introduction

In the rat ventral prostate (VP), castration is known to induce a marked reduction of prostatic epithelial cell mass. Ninety percent of the epithelial cells are lost within 8 days of castration [19]. Removal of the trophic hormone testosterone induces a cascade of events in prostatic epithelial cells that eventually results in apoptotic cell death [10, 19, 26, 38]. The percentage of epithelial cells dying per day through apoptosis is slightly increased at 1 day after castration, it is markedly so 2 to 5 days and only slightly so 7 days after castration [6]. The topographical distribution of apoptosis in the rat VP is apparently heterogeneous. It is most pronounced in the glandular parts most distal from the urethra [39]. The androgen receptor is, however, evenly distributed and there are no differences in receptor-binding activity, or 5α-reductase activity between the distal and proximal parts of the ducts [30]. This suggests that other local factors could be of importance for the induction of apoptosis in the VP. In other androgen-sensitive organs, such as the dorsal prostate (DP) and the coagulating gland, castration only marginally increases apoptotic cell death [1] and in the androgen-sensitive Dunning R3327-PAP tumor, the number of apoptotic cells even decreases after castration [43]. This suggests that the local environment of the androgen-sensitive cells may determine whether castration induces apoptosis or not.

Interestingly, apoptosis may, in mice prostatic tumor models [41] and in other organs such as brain, kidney and liver, be induced by low-grade or transient ischemia [22, 25, 31, 35, 46]. Previous studies have shown that VP blood flow is markedly reduced several days after castration [12] and it has also been suggested that prostatic blood vessels contain androgen receptors [29]. The aim of this study was, therefore, to examine if castration influences blood flow to the different prostatic lobes of the rat and to the Dunning 3327-PAP tumor.

Materials and methods

Animals and treatment groups

Male Sprague-Dawley rats were purchased from Möllegaard, 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 (fluanisonum 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 × 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 cm^3.

Blood flow measurements

Rats were anesthetized with a 1:1:2 mixture of Hypnorm (fluanisonum 10 mg/ml and fentanylum 0.2 mg/ml, Janssen Pharmaceutica), Dormicum (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 15.5 ± 0.1μm, Dupont Biotechnology, Wilmington, Del.) labeled with ^{141}Ce. The method used was originally described by Rudolf and Heymann [32] and modified by Damber and Janson [14, 15]. Manual vortexing was done directly in 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 $ml \times min^{-1} \times 100\ g^{-1}$.

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 × 5 min in citrate buffer using an antigen retrieval method as earlier described [38]. The sections were put in 3% H_2O_2 in methanol for 20 min and rinsed in PBS with 0.1% bovine serum albumin (BSA; Sigma). Normal 5% goat serum was used to minimize unspecific binding. The sections were then incubated overnight at 4°C with rabbit androgen receptor IgG (Biogenesis, N.H.) diluted 1/30. Localization of antibody-antigen complex was performed using the ABC (avidin-biotin complex) technique (Vector Laboraties, 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 nonparametric 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

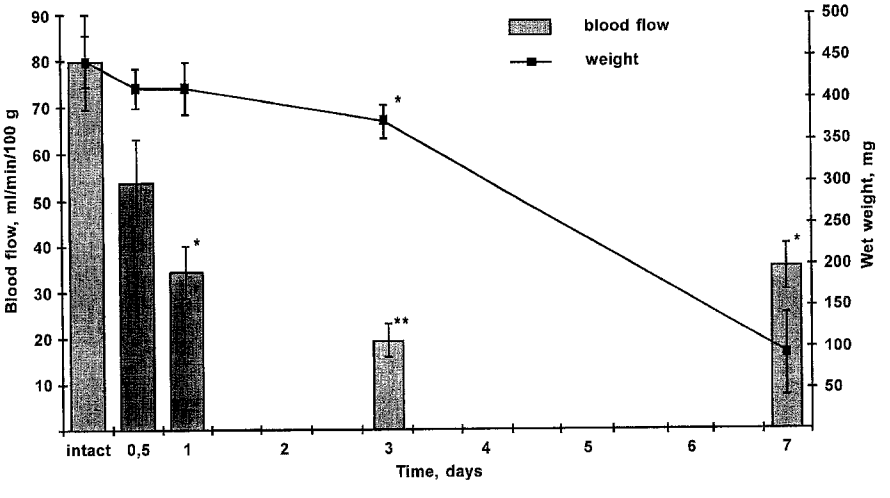

Fig. 1 The effect of castration on blood flow to, and wet weight of, the ventral prostate. Blood flow had already decreased significantly at 1 day, whereas the weight did not decrease until 3 days after castration. Values are means ± SEM. * $P < 0.05$, ** $P < 0.01$, when compared with intact animals (Mann-Whitney *U*-test)

Table 1 Blood flow and wet weight of different organs after castration. Mean values are given ±SEM; *n* number of observations within each group, *MAP* mean arterial pressure, *VP* ventral prostate, *DP* dorsal prostate, *ns* not studied

Group	*n*	MAP (mmHg)	Blood flow (ml × 100g^{-1} × min^{-1})			Wet weight (g)	
			Kidney	VP	DP	VP	DP
Intact	18	95 ± 3	512 ± 60	66 ± 7	34 ± 8	0.40 ± 0.02	0.21 ± 0.02
12 h[a]	9	104 ± 6	750 ± 96	58 ± 9	ns	0.40 ± 0.02	ns
1 day[a]	21	96 ± 3	480 ± 54	39 ± 5**	38 ± 9	0.40 ± 0.03	0.17 ± 0.02
3 days[a]	21	84 ± 2**	484 ± 48	22 ± 3***	23 ± 6	0.32 ± 0.02*	0.15 ± 0.02
7 days[a]	8	84 ± 4**	550 ± 63	46 ± 9*	34 ± 8	0.11 ± 0.03***	0.07 ± 0.02*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with intact animals
[a] Time after castration

Table 2 Blood flow to the proximal and distal parts of the VP at different times after castration. Mean values are given ± SEM; *n* number of observations within each group. None of the values for the distal VP are significantly different from the proximal VP when tested with the Mann-Whitney *U*-test.

Group	*n*	MAP	Blood flow (ml × min^{-1} × 100 g^{-1})	
			Proximal VP	Distal VP
Intact	6	98 ± 6	51.7 ± 15	57.4 ± 14
Day 1[a]	8	88 ± 2	20.9 ± 6	25.3 ± 6
Day 3[a]	12	80 ± 3	21.5 ± 2	28.3 ± 4

[a] Time after castration

Table 3 Ventral prostatic blood flow in intact, day-1, -3 and -7 castrated rats after testosterone supplementation at different times before measurements. Mean values are given and ±SEM; *n* number of observations within each group, *testo* testosterone

Group	Blood flow (ml × min^{-1} × 100 g^{-1})					
	Castration only		Castration + testo 8 h		Castration + testo 24 h	
Intact	66 ± 7	*n* = 18	65 ± 11	*n* = 7	ns	
Day 1	39 ± 5	*n* = 21	48 ± 6	*n* = 7	ns	
Day 3	22 ± 3	*n* = 14	36 ± 5*	*n* = 11	48 ± 17**	*n* = 7
Day 7	46 ± 9	*n* = 7	101 ± 9**	*n* = 6	62 ± 11	*n* = 5

*$P < 0.05$ and
**$P < 0.01$ when compared against the VP blood flow of animals in the castrated-only group

days (Fig. 1). VP blood flow was significantly ($P < 0.05$) decreased 1 day after castration, and remained low until 7 days after castration (Fig. 1, Table 1). There was no significant difference in the blood flow decrements between the distal and proximal parts of the VP (Table 2). There was a tendency for flow to decrease at 12 h that did not reach statistical significance. The blood flow rates to the kidneys and the DP were largely unaffected by castration (Table 1). Testosterone given 8 or 24 h before measurements in 1- and 3-day castrated rats increased blood flow in a dose-related way, but it could not normalize the blood flow rate in the VP at day 3 (Table 3). In contrast, testosterone treatment induced a major and rapid increase in VP blood flow in 7-day castrated rats. (Table 3, Fig. 2) Castration did not affect Dunning tumor blood flow (Table 4).

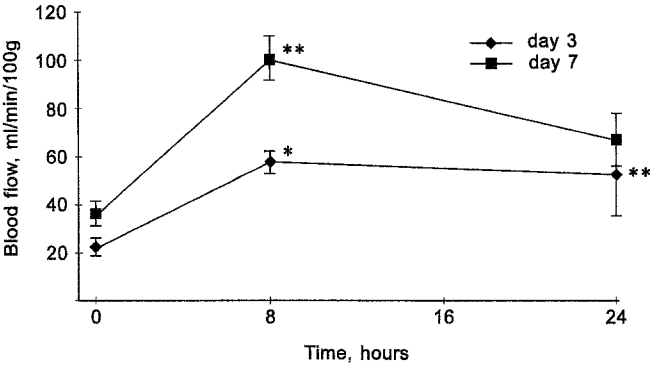

Fig. 2 Blood flow to the ventral prostate after castration with or without testosterone substitution. Substitution with testosterone at 8 and 24 h before blood flow measurements showed a marked response at 7 days only. Values are means ± SEM. *$P < 0.05$, **$P < 0.01$, when compared with animals without testosterone substitution (Mann-Whitney *U*-test)

Table 4 Blood flow to VP and Dunning tumor after castration. Mean values are given ± SEM; *n* number of observations within each group

Group	*n*	MAP	VP blood flow	Tumor blood flow	Kidney blood flow
Intact	8	78 ± 3	51 ± 12	81 ± 13	409 ± 40
3 days after castration	10	70 ± 3	33 ± 5*	85 ± 12	455 ± 93

*$P < 0.05$ when compared with the intact group

Androgen receptor immunohistochemistry

In the intact VP, DP and Dunning tumor immunoreactive androgen receptors (AR) were observed in the nuclei of the glandular epithelial and stroma cells, but receptors were not found in the endothelium or smooth muscle layer of blood vessels (Fig. 3). Castration did not influence the cellular distribution of AR, but the staining was much weaker, as shown by Prins [28] (not shown). As a positive control of vascular staining, androgen receptors were observed in testicular blood vessels, as earlier described [3] (not shown).

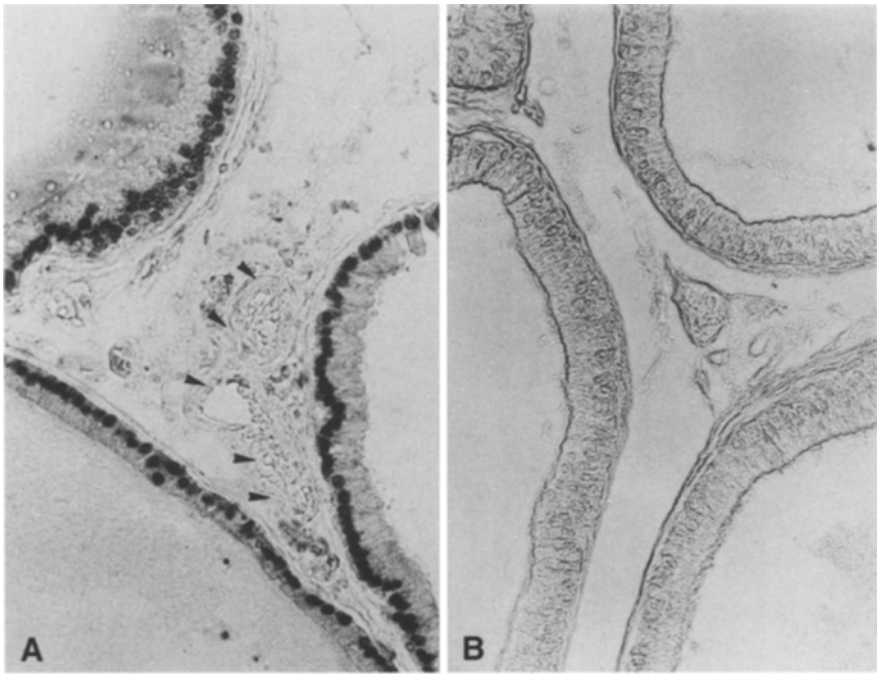

Fig. 3 A A section from the ventral prostate immunostained to visualize androgen receptors (×400). Such receptors as are in this lobe are observed principally in nuclei of epithelial cells, but not in blood vessels (*arrowheads*). **B** A control section incubated with androgen receptor-antiserum neutralized with excess of the immunogen is unstained (×400)

Discussion

This study demonstrates that castration induces a rapid decrease in blood flow to the VP. The fall in blood flow precedes the morphological apoptotic cell death that peaks at 2 to 3 days after castration [6] as well as the decrease in organ weight. There was, however, no significant decrease in blood flow to the DP or Dunning tumors after castration. Interestingly, recent studies have shown that castration does not induce apoptotic cell death in those tissues [1, 43]. Hypothetically, the absence of a castration-induced decrease in blood flow might thus be one reason why castration fails to induce apoptosis in DP [1] and in the Dunning tumors, a tumor that probably originated in the DP [17]. In several other organs such as kidney, liver and brain, apoptosis may be induced by low grade or transient ischemia [22, 25, 31, 33, 35, 46]. It is therefore possible that the major decrease in blood flow to the rat VP induces low-grade ischemia and that this might be of importance for the induction or potentiation of the apoptotic process and consequently for the rapid castration-induced involution of the VP.

The distribution of apoptosis in the VP after castration is heterogeneous in that it is reported to be maximal in the parts most distal from the urethra [39]. If blood flow is an important factor for the induction or potentiation of an apoptotic response, this distribution would be the expected one, since the principal arteries supporting the VP enter at the base [5] and end at the distal parts of the glands. However, we have not been able to show any difference in regional blood flow to the VP after castration (Table 4).

If reduction in blood flow is a necessary component in a full-scale apoptotic and involution response in androgen-dependent organs it would obviously be of importance to know the factors regulating blood flow in the normal and malignant prostate. In other endocrine or steroid sensitive organs, such as testis, adrenal cortex, ovary, and uterus, blood flow is regulated by the stimulatory hormone, suggesting a relation between function and blood flow [4, 8, 11, 18, 36, 40, 42]. At menstruation, uterine blood flow decreases, which leads to hypoxia, apoptosis and necrosis [37].

Prostate blood flow is regulated by general factors such as blood pressure and body temperature [20]. Previous work by our group has reported both lower [12, 15] and similar [16, 34, 44, 45] values of prostatic blood flow compared with the present study. It is our experience that systemic blood pressure is of importance for the absolute level of prostatic blood flow, since it is most likely that the prostate gland lacks autoregulation. It is also influenced by hormones, most importantly by testosterone [13, 34]. Long-term withdrawal of testosterone decreases blood flow, and testosterone substitution maintains a normal blood flow to the VP in castrated animals. Other steroid hormones also have an effect on blood flow to the prostate. For example, estrogen decreases blood flow in experimental animals and in humans [13, 26].

Both the short-term, as in the present study, and long-term castration-induced decreases in blood flow could be the result of a general effect of decreased metabolism, but it could also be caused by stimulation or inhibition of a specific vasoregulatory factor. Interestingly, potent vasoconstrictors such as endothelin-1 [24] and vasodilators like nitric oxide [7] are produced

locally in the prostate and possibly regulated by androgens [9]. A direct effect of androgens on the vasculature is one possibility, as other authors have suggested that vascular AR are present in the VP [29]. In this study such receptors were not observed, suggesting that the effect of testosterone on prostatic blood flow is mediated via effects on other cells.

In the present study we demonstrated that the VP blood flow is partly refractory to testosterone substitution during the early phase of castration-induced involution, but the reason for this remains unknown. Interestingly, castration leads to a decrease in the endothelial cell population in the VP at 7 days after castration [19]. It remains unclear whether the endothelial cell death is a result of the general involution of the gland or if there is a specific mechanism responsible for it.

Conclusion

This study clearly shows that a significant decrement in blood flow to the VP after castration takes place before there is any decrease in wet weight. There is no significant decrement in blood flow to the DP or Dunning tumor. This indicates that the decrease in prostatic blood flow might be a factor involved in the apoptotic process in the prostate after castration.

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